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IgE and Anti-IgE Therapy in Asthma and Allergic Disease

edited by
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IgE AND ANTI-IgE THERAPY IN ASTHMA AND ALLERGIC DISEASE

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INTRODUCTION

In their Preface, the editors of this volume describe the IgE/anti-IgE story as an exciting one. An exciting and fascinating story it is indeed.

Since the publication of the first volume in the Lung Biology in Health and Disease series, many texts on asthma have appeared. Although asthma is not a new disease, we can safely say that its story has been virtually rewritten over the past 20 years. New observations and discoveries, as well as the reassessment of old ones, have led to the development of these many monographs. In addition, the pace of advances has been remarkable. As happens with other diseases, the drive for these advances has certainly been the research opportunities from which the community of scientists has benefitted; however, in the case of asthma, the ever-increasing public health burden that this disease represents has greatly increased the impetus to do more, and do it faster!

And indeed, during the past two or three decades, we have seen astonishing progress. The development and use of new, more potent bronchodilators first dominated the therapeutic landscape. Then the realization that asthma is an inflammatory disease led to the greater use of anti-inflammatory drugs, with glucocorticosteroids in the vanguard. But, as research uncovered the inflammatory cascade, from the insult to the response, new agents capable of interrupting the

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cascade were discovered and used very successfully. One such example is antileukotrienes.

Yet, long before this remarkable era, the IgE molecule had been recognized and its role defined by the Ishizakas. In fact, their research on the IgE molecule was one of the first steps, if not the very first, to establish asthma as an inflammatory disease.

In the mid-1970s, the first major clinical studies demonstrated the therapeutic significance of anti-IgE molecules. Then, more recently, the ''neutralization'' of the IgE molecule reached new peaks with the availability of the recombinant, humanized monoclonal anti-IgE antibody. Today, we have in hand the results of many clinical trials, especially in children, that establish the value of this new pharmacological agent. And therein lies the excitement of this story.

The new monograph, edited by Robert Fick, Jr., and Paula Jardieu, reports the story from the basics to the application. The authors as well as the editors are the researchers and clinicians who developed most of the story. So, we have it firsthand!

The Lung Biology in Health and Disease series benefits greatly from the inclusion of this particular volume. As executive editor of the series, I am grateful to all the contributors for giving me the opportunity to present this new volume. However, it is the patients who will benefit the most from the work reported here.

Claude Lenfant, M.D.
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FOREWORD

Although today the IgE immune effector system is well established and the role of IgE antibodies in allergic disease is widely appreciated, this was not the case as recently as 30 years ago. We have progressed a great distance in a relatively short span on the timeline of science. When we initiated our studies on human reaginic antibodies, the majority of immunologists and allergists believed that these antibodies belonged to IgA because of the work by Heremans and Vaerman in 1962 (1). They demonstrated that the reaginic activity in the serum of allergic patients was recovered in the IgA fraction, in which no other serum protein was detectable. In 1963–64 their data were reproduced by several investigators and evidence accumulated to support the IgA hypothesis (2,3). We became suspicious about the concept, however, because of findings showing that reaginic activity in the ''pure IgA fraction'' could not be removed by precipitation of IgA with anti-IgA (4). Additionally, the human IgA isoagglutinin failed to sensitize human skin for Prausnitz-Küstner reaction (5). The results indicated then that reaginic activity should be associated with impurities present in the IgA fraction, and further, that the serum concentration of any unique immunoglobulin with which the reaginic activity is associated must be extremely low compared with the other

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known immunoglobulin isotypes. These findings forced us to change our strategy from isolation of the relevant IgA fraction to identification of the novel immunoglobulin.

Retrospectively, the tool that provided the key to identification of IgE was the preparation of polyclonal antibodies specific for IgE. Using the antibodies, reagin could be identified in vitro and IgE could eventually be isolated from the serum of atopic individuals (6). The anti-IgE antibodies were also employed to identify the first and second cases of IgE myeloma proteins (7) and to establish that IgE represents a unique immunoglobulin isotype (8).

When IgE was identified, a majority of allergists were suspicious about the role of reaginic antibodies in allergic diseases. However, somewhat more convincing to them was the fact that we could demonstrate the reaction of either allergen or anti-IgE to cell-bound IgE causing the release of histamine and leukotrines (9,10). These findings caught the clinicians' attention. Subsequently, we succeeded in identifying the target cells for IgE as basophilic granulocytes and mast cells (11) and obtained experimental evidence that structures in the Fc portion of IgE are involved in passive sensitization of target cells (12). The presence of IgE as a unique immunoglobulin isotype was confirmed and the role of IgE antibodies in allergic disease was on its way to becoming established. The wonderful science of monoclonal anti-IgE in the 1990s has made possible confirmation and then extension of the earliest reports in the 1960s and 1970s.

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References

1. Heremans JF, Vaerman JP. Nature 1962; 193:1091.
2. Ishizaka T. Mechanisms of IgE-mediated hypersensitivity. In: Middleton E, Jr., Reed CE, Ellis EF, Franklin Adkinson N, Jr., Yunginger JW, eds. Allergy Principles and Practice. St. Louis: Mosby 1988:71–93.
3. Yount WJ, Dorner MD, Kunkel HG, Kabat EA. Studies on human antibodies. J Exp Med 1968; 127:633–646.
4. Ishizaka K, Ishizaka T. Physicochemical properties of reaginic antibody. Association of reaginic activity with an immunoglobulin other than gammaA- or gammaG-globulin. J Allergy 1966; 37(3):169–185.
5. Ishizaka K, Ishazaka T, Lee EH. Physicochemical properties of reaginic antibody: characteristic properties of reaginic antibody different from human gamma-A-isohemagglutinin and gamma-D-globulin. J Allergy 1966; 37(6):336–349.
6. Ishizaka K, Ishizaka T, Hornbrook MM. Allergen-binding activity of gamma-E,

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gamma-G and gamma-A antibodies in sera from atopic patients: in vitro measurements of reaginic antibody. J Immunol 1967; 98(3):490–501.
7. Ogawa M, Kochwa S, Smith C, Ishizaka K, McIntyre OR. Clinical aspects of IgE myeloma. N Engl J Med 1969; 281(22):1217–1220.
8. Ishizaka K, Ishizaka T, Hornbrook MM. Physicochemical properties of reaginic antibody. Correlation of reaginic activity with gamma-E-globulin antibody. J Immunol 1966; 97(6):840–853.
9. Ishizaka T, Ishizaka K, Orange RP, Austen KF. The capacity of human immunoglobulin E to mediate the release of histamine and slow reacting substance of anaphylaxis (SRS-A) from monkey lung. J Immunol 1970; 104(2):335–343.
10. Ishizaka T, Ishizaka K, Johansson SG, Bennich H. Histamine release from human leukocytes by anti-gamma E antibodies. J Immunol 1969; 102(4):884–892.
11. Helm B, Kebo D, Vercelli D, Glovsky MM, Gould H, Ishizaka K, Geha R, Ishizaka T. Blocking of passive sensitization of human mast cells and basophil granulocytes with IgE antibodies by recombinant human epsilon-chain fragment of 76 amino acids. Proc Natl Acad Sci USA 1989; 86:9465–9469.
12. Ishizaka T, Helm B, Hakimi J, Niebyl J, Ishizaka K, Gould H. Biological properties of a recombinant human immunoglobulin epsilon-chain fragment. Proc Natl Acad Sci USA 1986; 83(21):8323–8327.

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PREFACE

This volume chronicles the successes of a true ''bench-to-bedside'' story. The storyline moves logically from the identification of an antiserum precipitating reaginic activity (termed ''IgE'' in part because when injected it caused skin erythema) through well-controlled clinical trials 25 years later demonstrating the safety and efficacy of an anti-IgE monoclonal antibody in allergic disease. Biomedical scientists had labored for three decades to block IgE interaction with immune effector cells expressing IgE receptors. The engineering of a humanized recombinant monoclonal antibody* binding IgE at an epitope shared by IgE receptors FcεRI and FcεRII has provided researchers interested in the immunopathogenesis of asthma with a safe and effective probe uniquely suited to delin-

* A word concerning consistency of reference to the monoclonal anti-IgE molecules discussed in this volume: it is the murine antibody MAE11 that was humanized and version 25 (E25 or rhuMAb-E25) was selected for clinical studies. As patient safety was demonstrated and clinical successes mounted, the laboratory designation ''E25'' was assigned new names (omalizumab/Xolair). The term ''anti-IgE'' is intended to refer more broadly or generically to the concept of antibodies binding IgE. Another murine antibody, TES-C21, was humanized and in limited early clinical development is referred to as hu56901 (or, in the early literature, as CGP56901).

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eate the contribution of IgE. Beyond asthma, anti-IgE will someday also provide invaluable insights into the role of IgE in seasonal and perennial allergic rhinitis, atopic dermatitis, helminth parasitic infections, and allergic bronchopulmonary aspergillosis—to mention only a few disorders discussed in the chapters that follow.

To be sure, technological and conceptual breakthroughs were needed before the fruits of clinical activity in important allergic diseases could be enjoyed and the dream realized. Köhler and Milstein showed us how to immortalize antibody-producing cells from a mouse spleen and thereby reliably produce large quantities of individual (monoclonal) antibody. In recent years there has been spectacular growth in the use of MAbs to study the role of inflammatory mediators in experimental asthma, and currently there are 10 MAb therapeutics approved by FDA providing important treatments for diseases as varied as Crohn's disease, respiratory syncytial virus infection, and breast cancer. Certainly the early reports (1967–70) describing the role of the new immunoglobulin (IgE) in allergic disease, and later the identification of the domains on IgE responsible for IgE attachment to immune effector cells, were seminal observations. Additionally, the engineering of an IgE-binding MAb that did not crosslink IgE bound to the surface of high-affinity receptor bearing cells, thereby creating a nonanaphylactogenic antibody, was a conceptual breakthrough essential to clinical studies in patients with allergic disease. Ultimately, the ability to ''humanize'' antibodies derived from murine sources has made possible prolonged repeated administration in patients with chronic diseases.

This is an exciting story, but it would not appear before you without the contributions of a great number of professionals whose efforts have directly resulted in this volume in the prestigious Lung Biology in Health and Disease series. We wish, first and foremost, to recognize the chapter authors, selected as recognized international authorities, who have contributed outstanding scholarly reviews. Dr. Lenfant has been an unwavering champion of this volume. His help extends beyond this single volume to his encouragement of a career dedicated to lung research (RBF) supported in the 1980s by the NHLBI Lung Division's Clinical Investigator Award Program. Two Marcel Dekker, Inc., colleagues have made remarkable contributions. Sandra Beberman, Vice President, Medical Division, displayed a saintly level of patience during a prolonged period of writing, and Moraima Suarez, Production Editor, served as a confident guide through the complexities of the production of a textbook of this breadth. We would like to acknowledge the daily unflagging support of our Genentech Executive Assistants, sage partners managing challenging schedules. This volume could not have been completed without the assistance provided by Marilyn Anchick and Bernadette McAllister.

In closing, we wish to acknowledge our patients. Included are the 1.5 million U.S. asthmatics requiring urgent medical care for severe asthma exacerba-

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tions, the 500,000 occupying hospital beds, and the 5000 who continue to die annually from reversible obstructive lung disease. Sadly, we recognize that there are far greater numbers of patients who continue to awaken from sleep short of breath and patients who are forced by allergic disease to restrict their activities, narrow their choices, and fall short of their full potential. Finally, we recognize our patients who in the ultimate display of altruism have chosen to volunteer for clinical studies of Xolair. Without such selfless displays of faith and courage there would be no therapeutic advances with which to fill books.

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1

Introduction

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Allergy as a disease has a fairly decent historical tradition. In 1819 Dr. John Bostock reported to the Royal Medical and Chirurgical Society of London on his own medical problems of ''periodical affection of the eyes and chest,'' which he later called *Catarrhus aestivus* or ''hay fever.'' He attributed his symptoms, which occurred during the summer, to sunshine and warmth. In 1873 Charles Harrison Blackley first reported the techniques of nasal pollen provocation tests, scratch tests in the skin, and pollen counts obtained by a kite carrying a greasy glass plate. He established thus the basis for allergy practice, which remained substantially unchanged for nearly 100 years.

In 1919 a case was reported of asthma caused by allergy to horse dander, which had been passively transferred by blood transfusion (1), and in 1921 Prausnitz and Küstner (2) performed their famous passive transfer of a positive skin test, later called the PK test. Concurrently and independently, Dr. Arent de Besche in Norway was carrying out similar studies using serum from patients allergic to horses.

The term ''atopy'' was introduced by Coca and Cooke in 1923 (3). They reported the presence of heat-labile ''bodies,'' which they called ''reagins'' by virtue of their similarity in this respect to the heat-labile reagins (complement) of the Wassermann reaction. They concluded that it is ''advisable for the present

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to avoid the term 'antibodies','' because there was ''no evidence that these bodies appeared as the result of immunological stimulation.''

Despite great efforts during the next 40 years, very little progress was achieved with regard to isolation and characterization of the reagin. In retrospect, it is easy to understand why. The extremely low serum concentration and its great biological lability obscured most experimental data. As late as 1965, in a textbook on immunological diseases, it was speculated that the reaginic activity ''is not a single, indivisible molecular species but is present in allergic sera in the form of labile complexes'' (4) that ''differ radically from immune antibodies.''

In 1966–67, the Ishizaka group in Denver, Colorado, claimed to have an antiserum that could precipitate reaginic activity (5). It was postulated that this represented a new immunoglobulin, provisionally called γE-globulin. One reason for the name was that when injected into human skin the fraction caused erythema, a reaction that, ironically, is not IgE-mediated. Despite great effort, they never succeeded in purifying the γE-globulin, which is not surprising considering its extremely low serum concentration.

In 1965, independent of this work and for quite other reasons, Bennich and Johansson, in Uppsala, Sweden, detected an atypical myeloma protein that was found to share the physicochemical properties characteristic of the reagin (6). A normal counterpart, provisionally called IgND, could be detected in serum of healthy individuals, and patients with allergic asthma had on average a sixfold higher concentration of IgND than normals or patients with nonallergic asthma (7). A radioimmunoassay (RAST) was developed, capable of detecting IgND antibodies to allergen, which correlated well with skin testing (8). In collaboration with Denis Stanworth from Birmingham, United Kingdom, it was shown that the PK reaction in humans could be blocked in a dose-dependent way with isolated IgND (9) or Fc fragments of the ND protein (10).

It was found that γE-globulin and IgND shared antigenic properties. At a workshop in Lausanne in February 1968, in which the main researchers from the two groups participated, it was finally agreed that there were enough data available on the structure and antigenicity of the material to allow the declaration of a new immunoglobulin, which was called IgE (11). In addition, its relationship to the reaginic activity was confirmed. This finding confirmed that immunological intervention intended to eliminate or decrease symptoms of atopic allergy can only be effective if an IgE sensitization is present.

During the first two thirds of the twentieth century, many doctors and scientists regarded allergology as a suspicious specialty. This situation changed drastically once IgE was recognized. It then became possible to measure accurately the immunological sensitization by analyzing the presence of IgE antibody to almost any allergen.

Today, our knowledge about the role of IgE in allergic inflammation is substantial. According to the present hypothesis of how the immune system is

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controlled, there is a balance between Th_1 and Th_2 helper cells. Th_1 cells promote immune protection against bacterial and viral infections, and Th_2 cells protect the body from helminthic infestations and are perhaps also involved in maintaining pregnancy (12). The precise, beneficial role of IgE is still not known. However, both systems also have adverse reactions; Th_1 is involved in autoimmunity and allergic contact dermatitis and Th_2 in IgE-mediated allergy. It should be remembered that cells and mediators of the immune system are found in biopsies of any inflammatory site, as immunological reactions are of course involved in mediating and initiating inflammatory as well as allergic responses.

Thus, presence in the tissue or circulation of increased numbers of eosinophils, IL-5, or even polyclonal IgE without significant antibody activity does not necessarily indicate an allergic reaction. One example is the eosinophilic inflammation and polyclonal IgE response typical of nonallergic nasal polyps (13), probably driven by ''superantigens'' of *Staphylococcus aureus*. Similar mechanisms might explain the microscopic and immunological appearance of IgE in the bronchial mucosa in biopsies of nonallergic asthma (14).

Although we have a good understanding of IgE and allergic inflammation, we still do not understand enough of the primary sensitization reaction. This can be illustrated by any of the recent epidemiological investigations that show a steady increase in the prevalence of allergic diseases over the last 100 years or so. It is generally believed that ''lifestyle factors'' are of great importance to this increase, but until we understand, in some detail, the mechanisms of sensitization, it will be very difficult to interfere effectively with the aim of decreasing allergy.

In addition to classical treatment with pharmacologically active drugs, new treatments based on manipulation of the immune reaction are approaching completion. One example is injection of monoclonal antibodies specific to interleukins, e.g., IL-5 (15), which could inactivate eosinophils. Another is antibodies to IgE (16), which will decrease circulating and cell-bound IgE, hopefully leading to an amelioration of symptoms.

The introduction of a new drug, omalizumab, which is a humanized monoclonal antibody to IgE, has a most interesting potential to decrease or eliminate IgE molecules, irrespective of allergen specificity, from the circulation and from the surface of inflammatory cells. Thus, in IgE-mediated diseases this new treatment has the potential to be most effective. On the other hand, nonallergic inflammation or inflammation caused by non-IgE mechanisms cannot, by definition, be dampened by omalizumab. As a consequence, more attention must be paid to identification of the immunological sensitization than today when many physicians look at and treat symptoms with no regard to the underlying pathological mechanisms.

The idea of producing a reagent that could attack and eliminate the key player in the classical allergic reaction, IgE, is not new, and it has taken quite some time and effort to achieve the present outcome of the launch of a humanized

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antibody capable of fulfilling these expectations. By drastically reducing the amount of circulating IgE, which in itself is a remarkable task considering the short 2- to 3-day half-life of free IgE, the number of IgE molecules and its high-affinity receptors on mast cells and basophils are also reduced. As a result, the density of cell-bound IgE antibody with any allergen specificity is reduced below the threshold of molecules, 300 or so per cell, needed to trigger the cell to release its mediators.

What is the future for allergic individuals? Obviously, once readily available at reasonable cost, an anti-IgE treatment, like omalizumab, will have a significant impact on the need for allergen-specific diagnosis and treatment. The allergic patient deserves to know what is causing his or her symptoms. Only the patient can take full responsibility for his or her situation, e.g., by avoiding exposure to cats, or by not eating eggs, fish, or peanuts. However, the complicated, multisensitized, and multisymptomatic patients, who today represent a considerable burden on health care resources, can be managed simply through IgE elimination once it has been shown that their symptoms are IgE-mediated.

This volume in the Lung Biology in Health and Disease series is dedicated to summarizing our present knowledge of the mechanisms and use of IgE elimination in the treatment of IgE-mediated allergic diseases. Read it carefully. It represents a historical document. IgE was discovered 30 years ago and it may be eliminated from the world within another 30 years. Let us hope it is all to the benefit of the human race.

References

1. Ramirez MA. Horse asthma following blood transfusion: Report of a case. JAMA 1919; 73:984.
2. Prausnitz C, Küstner H. Studien über die Überempfindlichkeit. Zentralbl Bakteriol I Abt Orig 1921; 86:160.
3. Coca AF, Cooke RA. On the classification of the phenomena of hypersensitiveness. J Immunol 1923; 8:163–182.
4. Sehon AH, Gyenes L. Immunology of atopic diseases. Antibodies in nontreated patients and antibodies developed during treatment. In: Samter M, Alexander HL, eds. Immunological Diseases. London: J & A Churchill, 1965:519–538.
5. Ishizaka K, Ishizaka T. Identification of γE-antibodies as a carrier of reaginic activity. J Immunol 1967; 99:1187.
6. Johansson SGO, Bennich H. Studies on a new class of human immunoglobulins. I. Immunological properties. In: Killander J, ed. Gammaglobulins. Structure and Control of Biosynthesis. Stockholm: Almqvist & Wiksell, 1967:193–197.
7. Johansson SGO. Raised levels of a new immunoglobulin class (IgND) in asthma. Lancet 1967; ii:951–953.
8. Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an in-vitro test for allergen antibodies. Lancet 1967; ii:1105–1107.

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9. Stanworth DR, Humphrey JH, Bennich H, Johansson SGO. Specific inhibition of the Prausnitz-Küstner reaction by an atypical human myeloma protein. Lancet 1967; ii:330–332.
10. Stanworth DR, Humphrey JH, Bennich H, Johansson SGO. Inhibition of the Prausnitz-Küstner reaction by proteolytic cleavage fragments of a human myeloma protein of immunoglobulin class E. Lancet 1968; ii:17–18.
11. Bennich HH, Ishizaka K, Johansson SGO, Rowe DS, Stanworth DR, Terry WD. Immunoglobulin E, a new class of human immunoglobulin. Bull World Health Org 1968; 38:151–152.
12. Holt PG, Jones CA. Allergy Review Series VI: The immunology of fetuses and infants. The development of the immune system during pregnancy and early life. Allergy 2000; 55:688–697.
13. Bachert C, Gevaert P, Holtappels G, Johansson SGO, Van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. J Allergy Clin Immunol. In press.
14. Humbert M, Menz G, Ying S, Corrigan CJ, Robinson DS, Durham SR, Kay AB. The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: more similarities than differences. Review. Immunol Today 1999; 20:528–533.
15. Cuss FM. Therapeutic effects of antibodies to interleukin-5. Allergy 1998; 53:89–92.
16. Patalano F. Injection of anti-IgE antibodies will suppress IgE and allergic symptoms. Review. Allergy 1999; 54:103–110.

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2

Serum IgE Distributions in Normal and Asthmatic Subjects

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I. Introduction

Closely following the identification in 1967 of the new immunoglobulin IgE possessing reaginic activity, the biological role of IgE in atopic allergy became apparent. Almost immediately attempts were made to modulate IgE and, it was hoped, improve the condition of highly allergic individuals worldwide. Study reports of the causes for variation in serum IgE in normal populations and the distribution of IgE in allergic population samples fueled these nascent therapeutic efforts. Population-based studies of serum IgE have garnered increased attention with the recent publication of study results of a safe and effective monoclonal antibody–binding IgE (omalizumab/Xolair™).

This chapter reviews the literature on the distribution of serum IgE levels in normal and asthmatic populations in developed countries including the United States, Canada, Europe, Scandinavia, and New Zealand. First, IgE levels observed in population-based studies of normal, healthy individuals are examined. Factors associated with serum IgE levels such as age, gender, race/ethnicity, and smoking are briefly discussed. Second, reports on the IgE levels in asthmatic and allergic populations both with and without an additional allergic comorbidity are reported. The association of serum IgE with disease status is discussed. Third,

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this chapter presents the baseline serum IgE levels in subjects who participated in three clinical trials of a novel anti-IgE therapeutic agent (omalizumab/Xolair™). The baseline levels of IgE in subjects with allergic asthma (AA) are presented in the context of what we would expect in normal, healthy people and in patients with AA based on the current scientific literature.

II. IgE Levels in Normal, Healthy Subjects

Several population-based studies have examined the distribution of serum IgE levels in the general population (Table 1) (1–8). Data from these population-based studies report overall geometric mean levels of IgE in the general population ranging from approximately 20 to 40 IU/mL, with variation according to age, sex, and geographic distribution (Table 1). In one of the largest population-based studies of IgE levels in the United States, the Tucson Epidemiological Study, the geometric mean level of IgE in 2743 white, non–Mexican American children and adults aged 6–64 years was 32.1 IU/mL (1). Several additional studies measured serum IgE levels in convenience samples from specific populations; values from these studies are similar to those in Table 1.

Some studies have evaluated the serum levels of IgE in nonallergic or nonasthmatic subjects only (Table 2) (1,2,9–15). Geometric mean levels of serum IgE are typically lower in studies of nonallergic subjects compared to mixed pools of allergic and nonallergic subjects. For example, among allergen skin test–negative subjects in the Tucson Epidemiological Study, the geometric mean IgE levels were 22.9 IU/mL for men and 14.7 IU/mL for women (1), somewhat lower

Table 1 Geometric Mean Values and Distribution of IgE (IU/mL) from Studies of the General Populations of United States, Canada, Europe, Scandinavia, and New Zealand

Location		Ref.
Tucson, AZ		1
Total		
Geometric mean (*n*)	32.1 (2743)	
95th percentile	553.1	
Men		
Geometric mean (*n*)	42.3 (1241)	
95th percentile	744.4	
Women		
Geometric mean (*n*)	25.6 (1502)	
95th percentile	409.7	

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Table 1 Continued

Location		Ref.
Saskatchewan, Canada (included children)		3
Whites of European descent		
Geometric mean (*n*)	81.3 (819)	
North American Indians		
Geometric mean (*n*)	275.4 (275)	
Manitoba, Canada		2
Men		
Geometric mean (*n*)	23.4 (889)	
Women		
Geometric mean (*n*)	18.7 (925)	
Norway		4
Men		
Geometric mean (*n*)	17.8 (650)	
Women		
Geometric mean (*n*)	13.8 (618)	
Budapest, Hungary		5
Men		
Geometric mean (*n*)	64 (164)	
Women		
Geometric mean (*n*)	34 (154)	
Paris, France		6
Men		
Geometric mean (*n*)	34.2 (189)	
95% CI	1.42–822.1	
Copenhagen, Denmark		7
Total		
Geometric mean (*n*)	22.9 (593)	
95% CI	14.3–31.5	
Three regions in New Zealand		8
Total		
Geometric mean (*n*)	43.4 (718)	
95% CI	38.6–48.9	
Men		
Geometric mean	47.5	
95% CI	40.2–56.1	
Women		
Geometric mean	39.6	
95% CI	33.4–46.8	

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Table 2 Geometric Mean Values and Distribution of IgE (IU/mL) from Studies of Nonallergic Subjects

Location		Ref.
Southern United States: nonatopic whites		9
Geometric mean (*n*)	32 (425)	
Richmond, VA: nonatopics/nonasthmatics		10
Black		
Geometric mean (*n*)	320 (97)	
White		
Geometric mean (*n*)	123 (53)	
San Diego, CA: nonatopics/nonasthmatics		11
Men		
Geometric mean (*n*)	21.4 (113)	
Women		
Geometric mean (*n*)	13.5 (88)	
Tucson, AZ: allergen skin test–negatives		1
Men		
Geometric mean (*n*)	22.9 (758)	
95th percentile	316.5	
Women		
Geometric mean (*n*)	14.7 (905)	
95th percentile	188.8	
Manitoba, Canada: nonatopics/nonasthmatics		2
Men		
Geometric mean (*n*)	14.7 (334)	
Women		
Geometric mean (*n*)	12.1 (359)	

than the levels reported from the mixed allergic and nonallergic populations (42.3 IU/mL for men and 25.6 IU/mL for women) (1).

A number of factors have been shown to correlate with serum levels of IgE. Serum IgE levels are age-related, with peak levels occurring during childhood, usually between the age of 8–12 years, and typically decreasing thereafter (1,5,9,10,16–18). In adults, serum IgE levels are higher in men than in women (1,2,4,5,7,11,16,19), although differences by gender are not as well established in younger populations (Fig. 1).

In addition to gender, serum IgE may vary by ethnicity: there is evidence that serum IgE levels are higher in blacks than in whites (9,10,17) and some evidence that serum IgE levels are higher in North American Indians(18) (see Tables 1 and 2). It is not clear if differences among these racial and ethnic groups are due to genetic differences or to differences in environmental exposures. High

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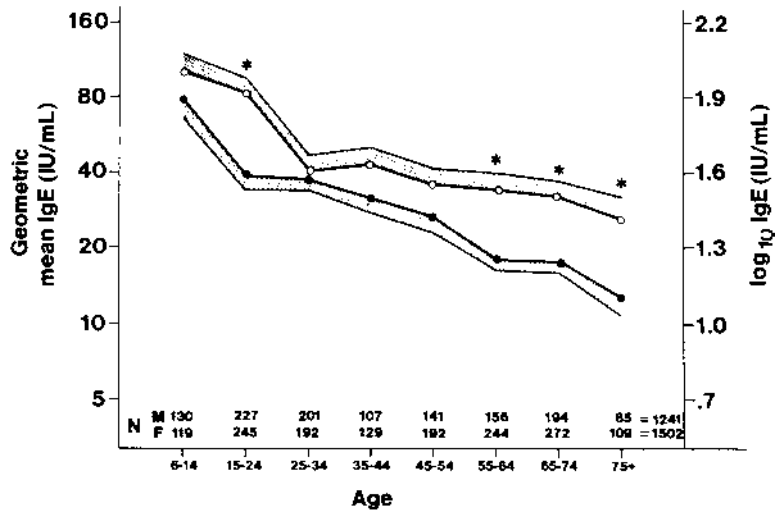

Figure 1 The distribution of IgE levels (IU/mL) by age and gender from the general population sample of the Tucson Epidemiological Study. (From Ref. 1.)

levels of serum IgE were observed in a study comparing serum IgE levels in North American Cree Indians relative to their white Saskatchewan counterparts. The authors theorized that these high IgE levels could be due to both unexplained genetic factors or environmental factors such as increased exposure to infections including more frequent attacks of gastroenteritis and bronchopneumonia in infancy, upper respiratory tract infections, and others. The inclusion of children in this study may partially explain the reported higher serum IgE levels compared to adult only populations.

Several studies have demonstrated that smokers have higher IgE levels than nonsmokers (4,11,20–22). However, the relationship between smoking and IgE levels has not been clearly established. In one study, serum IgE levels were not significantly different among nonsmokers, ex-smokers, and current smokers; however, they were significantly higher in heavy smokers (23). Others report that serum IgE levels were not correlated with either the amount smoked or the number of pack-years (21).

III. IgE Levels in Asthmatic and Allergic Populations

On average, asthma patients have higher IgE levels than healthy control patients (3,9–11,14,24,25), but there is considerable overlap in the distribution of IgE

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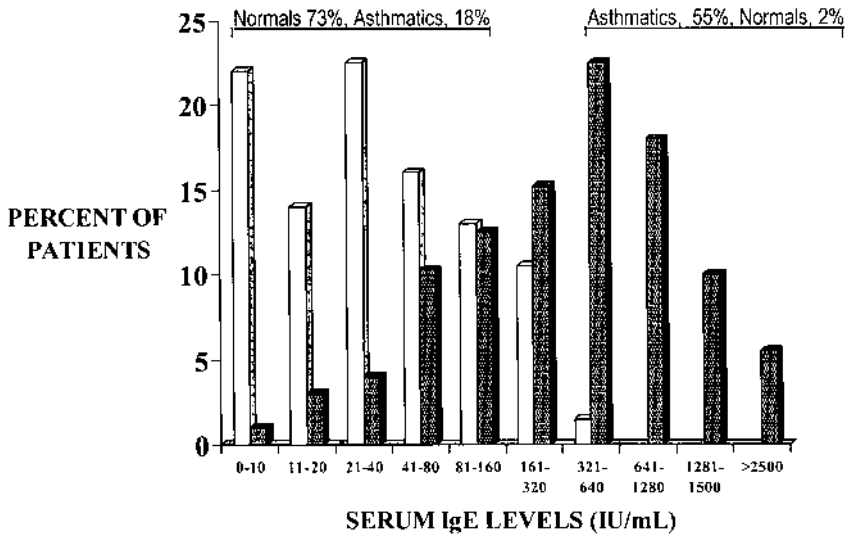

Figure 2 The distribution of IgE levels (IgE) in normal subjects and in subjects with asthma from a sample of the general population in the southern United States. Normals (□), N = 435; Asthmatics (■), N = 487. (Adapted from Ref. 9.)

among normal and asthmatic populations (9) (Fig. 2 and Table 3). In the Tucson Epidemiological Study, mean IgE levels were higher in all age strata of asthmatic patients compared to those without asthma. In multivariate analyses, serum IgE levels independently predicted asthma after adjustment for age, sex, smoking status, and skin test index (12). In the data from the Tucson Epidemiological Study, it appeared that asthma was almost always associated with a higher IgE level, thus challenging the concept that there are allergic and nonallergic forms of asthma (12).

In addition to results from the Tucson Epidemiological Study, other studies from diverse geographical areas have reported higher serum IgE levels in adults with asthma including San Diego, CA (11), Manitoba, Canada (2), South Wales (26), Charlottesville, VA (27,28), Richmond, VA (10), Portland, OR (21), Paris, France (23,24), Tucson, AZ (1,12), the southern United States (9), Haren, the Netherlands (29), Spain (30), Melbourne, Australia (31), and Dunedin, New Zealand (13). As in the general population data and in nonasthmatic populations, serum IgE levels increase with age in asthmatic or rhinitic children, peaking at approximately age 10 and then declining (12,32).

Studies in children with asthma confirm that serum IgE levels are higher than in nonasthmatic children. In children aged 2–16 years, geometric mean titers

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Table 3 Geometric Mean Values and Distribution of IgE (IU/mL) in Adults with Asthma, with or Without Allergic Disorders

Location		Ref.
Richmond, VA		10
Blacks with asthma		
Geometric mean (*n*)	678 (69)	
Whites with asthma		
Geometric mean (*n*)	219 (22)	
San Diego, CA: age 38–82 years		11
Men with asthma		
Geometric mean (*n*)	70.8 (17)	
Women with asthma		
Geometric mean (*n*)	47.9 (34)	
Southern United States		9
Asthma, no atopy	305 (507)	
Geometric mean (*n*)		
Asthma and atopic dermatitis	985 (48)	
Geometric mean (*n*)		
Paris, France: age 22–55 years		24
Men with asthma		
Geometric mean (*n*)	87.4 (23)	
Copenhagen, Denmark: age 14–71 years		14
Asthma and perennial rhinitis		
Median (*n*)	360 (53)	
Alberta, Canada: age 11–82 years		25
Asthma with negative allergen skin tests		
Log-mean (*n*)	60.2 (21)	
Asthma with positive allergen skin tests		
Log-mean (*n*)	172.3 (51)	
Saskatchewan, Canada: age 1–75 years		3
Asthma in whites of European descent		
Geometric mean (*n*)	210 (38)	

of serum IgE ranged from 99 to 548 IU/mL in those with asthma (9,19,33), compared with a range of 16.2–51 IU/mL in nonasthmatic or nonatopic children (1,9,34,35). In addition, there is some evidence that a younger age of onset of asthma may be associated with higher serum total IgE level as an adult (36).

A. IgE Levels in Asthma in Combination with an Additional Allergic Comorbidity

Several studies demonstrate that the highest serum IgE levels are found in patients with both asthma and an additional allergic disorder (2,9,14,25,37–40). In a study

of patients from the southern United States, the geometric mean IgE level was 985 IU/mL in patients with atopic dermatitis and asthma compared with 305 IU/mL in patients with asthma alone, 273 IU/mL in patients with atopic dermatitis alone, and 171 IU/mL in patients with allergic rhinitis (9). Similarly, in a study from Montpellier, France, median IgE concentrations were higher in patients with both atopic dermatitis and asthma than in those with asthma alone (40). Data from Switzerland also suggest that serum IgE levels are higher in patients with both atopic dermatitis and asthma than in those with atopic dermatitis alone (15).

B. Association of Serum IgE Levels with Disease Status in Subjects with Asthma

Several studies have found an association with serum IgE level and bronchial responsiveness in both asymptomatic subjects and in patients with a history of asthma (16,29,33,41–44). In a Spanish study of 214 adults with a past history of asthma, bronchial responsiveness in response to methacholine challenge showed a linear relationship with serum IgE levels (16). This relationship was strongest for patients with active asthma compared to those without symptoms. Other studies have also found an association between high serum levels of IgE and bronchial responsiveness to methacholine or histamine challenge (41,42). In a population-based study in Italy, the odds ratio for serum IgE level $\geq$ 150 kU/L with bronchial reactivity to methacholine challenge was 3.18 after adjustment for age, sex, smoking status, asthma symptoms, and pulmonary function; in this study, only IgE level and atopy were found to independently predict bronchial responsiveness (42). Two studies from the Netherlands found a similar relationship between bronchial responsiveness and serum IgE levels in patients with asthma, particularly in nonsmokers (29,43).

The relationship between serum IgE level and lung function has also been explored, in both samples of healthy patients and in those with asthma or obstructive lung disease. Cross-sectionally, total serum IgE level was found to be inversely related to age- and height-adjusted FEV_1 score in 310 French adult men, particularly in subjects who had never smoked (24). Similarly, an inverse relationship between log IgE and FEV_1 was found in 1078 men in the Normative Aging Study (45). However, no relationship was found between log IgE levels and FEV_1/FVC in same study (45) or in the pooled results from two cohort studies in Portland, Oregon, of 863 adults (21). Several studies have examined the relationship between serum IgE levels and pulmonary function in patients with asthma (33,46,47). Thus, some studies report an association between IgE level and lung function while others do not. It may be that the findings vary according to the spirometric measure evaluated or other undetermined factors.

Additionally, several studies have examined the predictive value of serum IgE levels on lung function in subjects followed longitudinally. In 310 French

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men, there was a significant inverse relationship between serum IgE level and 5-year FEV_1 decline observed in nonsmokers and ex-smokers but not current smokers (24). In the 1078 men in the Normative Aging Study, however, no relationship between log IgE level and annual change in measures of pulmonary function was demonstrated in either smokers or nonsmokers after adjustment for age, height, and baseline pulmonary function. Thus, serum IgE levels were not predictive of an accelerated rate of pulmonary function decline in middle-aged and older men in this study (45). In the Oregon cohort studies, serum IgE levels were not found to be predictive of rate of decline in lung function over a period of 9–11 years when adjusted for height, age, and sex (21).

Data exploring the relationship of serum IgE level with the clinical severity of asthma are limited, with conflicting results. In data from the Childhood Asthma Management Program, log serum IgE was positively correlated with both duration of asthma and with the mean daily albuterol puffs used for symptoms during the month preceding study enrollment in 1028 children with mild-to-moderate asthma (33). In a study from Victoria, Australia, of 10-year old asthmatic children, mean IgE concentration increased progressively as the severity of asthma increased (48). A study from Melbourne, Australia, which utilized the same severity grading scale, also found that mean serum IgE levels generally increased as severity increased (31). However, the investigators note the presence of wide variations and considerable overlap of the IgE distributions in the 315 asthmatic children and in the control group of 82 children (31). Again, this supports the concept that IgE alone cannot be used as a cutpoint to distinguish between patients with or without asthma. In contrast to the data from Australia, other studies have found no relation between serum IgE levels and the frequency of asthma attacks, severity of asthma attacks, or physician-assessed asthma severity (36,49).

Thus, serum IgE levels may correlate with severity of asthma, but the data are not consistent. There is some evidence to suggest that serum IgE level in children may predict those in whom asthma will persist. In a longitudinal study of 540 children by Sherrill et al., higher mean serum IgE levels in children less than 1 year old were associated with higher mean serum IgE levels at ages 6 and 11 years and persistent wheezing (50). More longitudinal data of IgE and asthma associated clinical outcomes are needed to more fully understand the relationship between IgE levels and severity of asthma.

IV. New Data from Clinical Trials

Three Phase III pivotal trials of a novel anti-IgE therapeutic agent enrolled adolescents and adults 12–75 years of age with moderate to severe persistent asthma and children 6–12 years of age with mild to moderate persistent asthma. The trials included patients who had a documented history of asthma for greater than

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one year, were skin test positive, and had a serum IgE level of at least 30 IU/mL. The lower bound for the IgE levels was close to the mean level of 32 IU/mL reported for normal, healthy patients from two of the largest epidemiological studies (1,9). Additionally, adults were required to have an IgE $\leq$ 700 IU/mL, and children were required to have an IgE $\leq$ 1300 IU/mL. Thus, the distribution of serum IgE levels from the anti-IgE pivotal clinical trial data is artificially truncated. Nevertheless, we found that the geometric mean values for IgE in patients with AA in the anti-IgE clinical trials are consistent with reported means for patients with asthma from the available literature.

In the pediatric trial, children 6–12 years of age had a geometric mean IgE level of 241 IU/mL (95% CI 219–265; $n = 334$). In the adolescent and adult trial of moderate to severe asthma conducted in the United States, the overall geometric mean for patients aged 12–75 years was 131 IU/mL (95% CI 122–140; $n = 525$). The overall geometric mean in adolescents and adults ($n = 546$) from a third clinical trial conducted both in North America and Europe was 157 IU/mL (95% CI 146–168; $n = 546$).

To examine the effects of age and sex, we pooled the data from the two pivotal trials in adolescents and adults (Table 4). We observed the typical association of age and IgE with higher geometric mean IgE levels in adolescents compared with adults. Adolescents 12–17 years of age had a geometric mean IgE level of 208 IU/mL (95% CI 177–245). Adults aged 18–44 years had a geometric mean IgE level of 149 IU/mL (95% CI 139–159), and adults aged $\geq$45 years had a geometric mean IgE level of 125 IU/mL (95% CI 115–136). These data are consistent with observations from the literature that IgE levels wane with age (1,9,10,12,20,23).

The effect of sex appeared to vary with age, with differences being more apparent in adults than in children or adolescents. In children, although females had a higher geometric mean value than males, the distributions were similar. In the pooled data, male and female adolescents had similar geometric mean IgE levels and distributions, though the sample size was limited. The geometric mean value for IgE in adult males was higher than in adult females. The difference between the means and distributions of IgE levels by sex was more apparent in older than in younger adults.

Our data are consistent with the literature that IgE levels are higher among asthmatics than in a normal, healthy population (10,12,19,51). In the anti-IgE clinical trials, the geometric mean levels of IgE were higher in children and adolescents than in adults and higher in adult males than adult females.

We were also able to observe the stability of IgE concentrations over time by tracking the IgE levels in placebo patients. Mean total IgE concentrations were generally stable in placebo patients in the pivotal AA studies. Geometric means of total IgE in adolescents and adults were within 5% of baseline values after 6 months of follow-up. Total IgE within-patient variability in patients with AA

Table 4 Baseline Data from Pivotal Phase III Trials in Children (n = 334: 231 males and 103 females) and in Pooled Adolescents and Adults with Allergic Asthma (n = 1071: 483 males and 588 females)

IgE (IU/mL)	Total	Male	Female
Age: 6–12 yr			
Geometric mean	241	238	248
95% CI	219–265	211–269	211–291
n	334	231	103
Age: 12–17 yr			
Geometric mean	208	208	208
95% CI	176–246	169–257	159–272
n	76	54	22
Age: 18–44 yr			
Geometric mean	149	152	146
95% CI	139–159	138–168	133–160
n	622	272	350
Age: 18+ yr			
Geometric mean	139	150	132
95% CI	132–147	139–162	123–141
n	995	429	566
Age: 45+ yr			
Geometric mean	125	147	111
95% CI	115–136	128–167	99–124
n	373	157	216

over time was a composite of both assay variability and underlying changes in the patients' IgE levels. The intrapatient CV for placebo patients, calculated on the log scale, was 25% (n = 330) and ranged between 21 and 29% among the baseline IgE subgroups, with no obvious systematic relationship. Similar within-patient variability was observed by sex and by racial subgroups. Interestingly, higher within-patient variability in total IgE levels was observed in children 6–11 years of age (34% CV; n = 83). The number of elderly patients (>64 years) was too small to make an assessment about variability. Overall, average total IgE concentration changed little over a one-year period in placebo patients with AA.

V. Conclusion

From the available literature, it is clear that serum IgE levels are elevated in patients with asthma compared to healthy, normal controls. Several factors have been reported to be increased in patients with asthma, while other factors have

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Table 5 Factors Associated with Serum IgE Levels

Factors consistently reported to be associated with increased serum IgE levels in subjects with asthma:
Age (children < 12 years)
Male gender (adults)
Presence of an additional allergic comorbidity
Bronchial responsiveness
Factors that may be associated with increased serum IgE levels in subjects with asthma:
African American or American Indian racial/ethnic background
Smoking
Disease severity
Duration of asthma
Factors reported to be inversely associated with serum IgE levels in subjects with asthma:
Age (adults)
Lung function

been reported to have an inverse relation to serum IgE levels (Table 5). The lowest levels of serum IgE are observed in nonallergic and nonasthmatic populations, and the highest levels are observed in patients with asthma in combination with another allergic condition. However, because of the large overlap that occurs in the distribution of IgE levels among normal and asthmatic patients, it is not possible to use IgE alone as a means of identifying patients with asthma. Furthermore, data on the association between IgE and measures of disease status and disease severity are somewhat limited. Our own data on the levels of IgE in our large clinical cohort of adolescents and adults with severe AA and in children with mild to moderate AA are consistent with reported levels in the literature. These data represent the largest clinical cohort data available on the distribution of IgE in allergic asthma.

References

1. Barbee RA, Halonen M, Lebowitz M, Burrows B. Distribution of IgE in a community population sample: correlations with age, sex, and allergen skin test reactivity. J Allergy Clin Immunol 1981; 68(2):106–111.
2. Holford-Strevens V, Warren P, Wong C, Manfreda J. Serum total immunoglobulin E levels in Canadian adults. J Allergy Clin Immunol 1984; 73(4):516–522.
3. Gerrard JW, Geddes CA, Reggin PL, Gerrard CD, Horne S. Serum IgE levels in white and metis communities in Saskatchewan. Ann Allergy 1976; 37(2):91–100.
4. Omenaas E, Bakke P, Elsayed S, Hanoa R, Gulsvik A. Total and specific serum IgE

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levels in adults: relationship to sex, age and environmental factors. Clin Exp Allergy 1994; 24:530–539.
5. Meretey K, Jakab A, Szilassy K, Medgyesi G. IgE levels in normal human sera and IgG preparations. Haematologia 1989; 3:151–159.
6. Oryszczyn M, Annesi I, Neukrich F, Dore M, Kauffmann F. Longitudinal observations of serum IgE and skin prick test response. Am J Respir Crit Care 1995; 151: 663–668.
7. Nielsen N, Menne T. The relationship between IgE-mediated and cell-mediated hypersensitivities in an unselected Danish population: The Glostrup Allergy Study, Denmark. Br J Dermatol 1996; 134:669–672.
8. D'Souza W, Lewis S, Cheng S, McMillan D, Pearce N, Town I, Rigby S, Skidmore C, Armstrong R, Ruthorford R. The prevalence of asthma symptoms, bronchial hyperresponsiveness and atopy in New Zealand adults. N Z Med J 1999; 112:198–202.
9. Wittig HJ, Belloit J, De Fillippi I, Royal G. Age-related serum immunoglobulin E levels in healthy subjects and in patients with allergic disease. J Allergy Clin Immunol 1980; 66(4):305–313.
10. Grundbacher FJ, Massie FS. Levels of immunoglobulin G, M, A, and E at various ages in allergic nonallergic black and white individuals. J Allergy Clin Immunol 1985; 75(6):651–658.
11. Criqui M, Seibles J, Hamburger R, Coughlin S, Gabriel S. Epidemiology of immunoglobulin E levels in a defined population. Ann Allergy 1990; 64:308–313.
12. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989; 320(5):271–277.
13. Sears R, Chow C. Serum total IgE in normal subjects and the influence of a family history of allergy. Clin Allergy 1980; 10:423–431.
14. Mygind N, Dirksen A, Johnsen NJ, Weeke B. Perennial rhinitis: an analysis of skin testing, serum IgE, and blood smear eosinophilia in 201 patients. Clin Otolaryngol Allied Sci 1978; 3(2):189–196.
15. Wuthrich B. Serum IgE in atopic dermatitis. Clin Allergy 1978; 8:241–248.
16. Sunyer J, Anto J, Sabria N, Roca J, Morell F, Rodriguez-Roisin R, Rodrigo J. Relationship between serum IgE and airway responsiveness in adults with asthma. J Allergy Clin Immunol 1995; 95:699–706.
17. Grundbacher F. Causes of variation in serum IgE levels in normal populations. J Allergy Clin Immunol 1975; 56:104–111.
18. Gerrard JW, Ko CG, Dalgleish R, Tan LK. Immunoglobulin levels in white and metis communities in Saskatchewan. Clin Exp Immunol 1977; 29(3):447–456.
19. Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. N Engl J Med 1991; 325(15):1067–1071.
20. Tollerud D, O'Connor G, Sparrow D, Weiss S. Asthma, hay fever, and phlegm production associated with distinct patterns of allergy skin test reactivity, eosinophilia, and serum IgE levels. Am Rev Respir Dis 1991; 144:776–781.
21. Vollmer W, Buist A, Johnson L, McCamant L, Halonen M. Relationship between serum IgE and cross-sectional and longitudinal FEV1 in two cohort studies. Chest 1986; 90:416–423.

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22. Warren CP, Holford-Strevens V, Wong C, Manfreda J. Relationship between smoking and total immunoglobulin E levels. Allergy Clin Immunol 1982; 69(4):370–375.
23. Orysczyn M, Annesi I, Neukirch F, Dore M, Kauffmann F. Relationships of total IgE level, skin prick test response, and smoking habits. Ann Allergy 1991; 67:355–358.
24. Annesi I, et al. Total circulating IgE and FEV1 in adult men. An epidemiologic longitudinal study. Chest 1992; 101:642–648.
25. Salkie M, Weimer N, Herbert F. Serum total IgE and allergen specific IgE in an adult asthmatic population. Diagnostic Immunol 1983; 1:72–74.
26. Burr ML, St. Leger AS, Bevan C, Merrett TG. A community survey of asthmatic characteristics. Thorax 1975; 30(6):663–668.
27. Pollart S, Chapman M, Fiocco G, Rose G, Platts-Mills T. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989; 83:875–882.
28. Scalabrin S, Bavbek S, Perzanowski M, Wilson B, Platts-Mills T, Wheatley L. Use of specific IgE in assessing the relevance of fungal and dust mite allergens to atopic dermatitis: a comparison with asthmatic and nonasthmatic control subjects. J Allergy Clin Immunol 1999.
29. Panhuysen C, Vonk J, Koeter G, Schouten J, van Altena R, Bleecker E, Postma D. Adult patients may outgrow their asthma. Am J Respir Crit Care Med 1997; 155: 1267–1272.
30. Sunyer J, Anto J Soriano J, Roca J. Total serum IgE is associated with asthma independently of specific IgE levels. The Spanish Group of the European Study of Asthma. Eur Respir J 1996; 9:1880–1884.
31. McNichol K, Williams H. Spectrum of asthma in children—II. Allergic components. Br Med J 1973; 4:12–16.
32. Neffen H, Crisci C. Correlation between serum IgA, secretory IgA and total serum IgE in asthmatic and rhinitic affected patients. Allergol Immunopathol 1986; 14(5): 413–418.
33. Zeiger R, Heller S. The development and prediction of atopy in high-risk children: follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance. J Allergy Clin Immunol 1999; 95:1179–1190.
34. Wright AL, Holberg CJ, Martinez FD, Halonen M, Morgan W, Taussig LM. Epidemiology of physician-diagnosed allergic rhinitis in childhood. Pediatrics 1994; 94(6 Pt 1):895–901.
35. Hansen L, Halken S, Host A, Moller K, Osterballe O. Prediction of allergy from family history and cord blood IgE levels. A follow-up at the age of 5 years. Pediatr Allergy Immunol 1993; 4:34–50.
36. Spittle B, Sears R. Bronchial asthma: lack of relationship between allergic factors, illness severity and psychosocial variables in adult patients attending asthma clinic. Psychol Med 1984; 14:847–852.
37. Laprise C, Boulet L. Asymptomatic airway hyperresponsiveness: a three-year follow-up. Am J Respir Crit Care Med 1997; 156:403–409.
38. Magnan A, Fourre-Jullian C, Jullian H, Badier M, Lanteaume A, Vervloet D, Char-

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pin D. Rhinitis alone or rhinitis plus asthma: What makes the difference? Eur Respir J 1998; 12:1073–1078.
39. Inouye T, Tarlo S, Broder I Corey P, Davies G, Leznoff A, Mintz S, Thomas P. Severity of asthma in skin test-negative and skin test-positive patients. J Allergy Clin Immunol 1985; 75:313–319.
40. Bousquet J, Coulomb Y, Arrendal H, Robinet-Levy M, Michel F. Total serum IgE concentrations in adolescents and adults using the Phadebas IgE PRIST® technique. Allergy 1982; 37:397–406.
41. Lebowitz MD, Bronnimann S, Camilli AE. Asthmatic risk factors and bronchial reactivity in non-diagnosed asthmatic adults. Eur J Epidemiol 1995; 11(5):541–548.
42. ECRHS. Determinants of bronchial responsiveness in the European Community Respiratory Health Survey in Italy: evidence of an independent role of atopy, total serum IgE levels, and asthma symptoms. Allergy 1998; 53:673–681.
43. Grol M, Postma D, Vonk J. Risk factors from childhood to adulthood for bronchial responsiveness at age 32–42 yr. Am J Respir Crit Care Med 1999; 160:150–156.
44. Croner S, Kjellman N. Natural history of bronchial asthma in childhood. Allergy 1992; 47:150–157.
45. Shadick N Sparrow D, O'Connor G DeMolles D, Weiss S. Relationship of serum IgE concentration to level and rate of decline of pulmonary function: the Normative Aging Study. Thorax 1996; 51:787–792.
46. Sherrill D, Lebowitz M, Halonen M, Barbee R, Burrows B. Longitudinal evaluation of the association between pulmonary function and total serum IgE. Am J Respir Crit Care Med 1995; 152:98–102.
47. Omenaas E, Bakke P, Eide G, Elsayed S, Gulsvik A. Total serum IgE and FEV1 by respiratory symptoms and obstructive lung disease in adults of a Norwegian community. Clin Exp Allergy 1995; 25:682–689.
48. Hogarth-Scott R, Howlett B, McNicol K, Simons M, Williams H. IgE levels in the sera of asthmatic children. Clin Exp Allergy 1971; 9:571–576.
49. Wilson N, Dore C, Silverman M. Factors relating to the severity of symptoms at 5 years in children with severe wheeze in the first 2 years of life. Eur Respir J 1997; 10:346–353.
50. Sherrill D, Stein R, Halonen M, Holberg C, Wright A, Martinez F. Total serum IgE and its association with asthma symptoms and allergic sensitization among children. J Allergy Clin Immunol 1999; 104:28–36.
51. Duff A, Pomeranz E, Gelber L, Price F, Farris H, Hayden F, Platts-Mills T, Heymann P. Risk factors for acute wheezing in infants and small children: viruses, passive smoke, and IgE antibodies to inhalant allergens. Pediatrics 1993; 92:535–540.

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3

Understanding the Binding of IgE to Its High-Affinity Receptor and to an Anti-IgE Antibody

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Antigen-specific IgE antibodies bind to the α subunit of a specific high-affinity tetrameric receptor complex, FcεRI (1), expressed on mast cells, basophils, Langerhans cells, and monocytes (2–7). IgE binds FcεRI via its Fc region (constant domains Cε2, Cε3, and Cε4) (2), and multivalent antigen binding by the IgE Fab domains results in crosslinking and aggregation of FcεRI-IgE complexes. FcεRI crosslinking initiates a cascade of intracellular signaling events that are in part regulated by FcεRI γ and β subunits (8–10) and that triggers the release of inflammatory mediators, cytokines (11), and chemokines. Inflammatory mediators produce the clinical symptoms of allergic rhinitis and asthma (12,13), while cytokines and chemokines may amplify the cellular immune responses that regulate the pathophysiology of allergic disease (14). IgE also binds to a low-affinity receptor (FcεRII, CD23) (15), a member of a family of Ca^{2+}-dependent (C type) carbohydrate-binding proteins, expressed on B lymphocytes and eosinophils. IgE binding to CD23 may elicit several diverse biological activities (16,17). Antigen-IgE immune complexes can bind to both FcεRII on B cells and FcεRI on monocytes and Langerhans cells to enhance antigen presentation in various lymphoid compartments and regulate T-helper cell pathways that regulate IgE synthesis. Because there is some evidence to suggest that the epitopes in the Fc region of IgE that bind to both FcεRII and FcεRI may overlap, the development of an IgE

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antagonist that blocks IgE binding to both receptors may have a significant effect on the immunoregulation of allergic disease.

Since only a small fraction of FcεRI receptor complexes on the surface of a cell must be occupied with IgE and crosslinked to initiate the degranulation reaction (18), the goal of developing a high-affinity and specific antagonist to FcεRI-IgE has been elusive. Early experimental approaches to this problem involved the identification of nonanaphylatogenic anti-IgE or anti-FcεRI monoclonal antibodies and the use of both IgE peptides and soluble recombinant fragments of IgE and FcεRI as competitive inhibitors of mast cell degranulation in vivo. Prior to the recent crystallographic data on IgE, FcεRI, and their complex (19–21), structure-function studies employing site-directed mutagenesis of both IgE and the FcεRI α-chain enabled researchers to narrow their focus to specific domains in either IgE or FcεRI (22–24). These structure-function studies, in concert with the crystal structures, may augment the development of leads derived from structure-based peptides (25,26) or screening of either small chemical or phage-based peptide libraries (27,28).

I. Defining IgE Epitopes That Interact with FcεRI

Early studies utilizing peptides and protein fragments derived from the sequence of IgE were designed to identify binding site(s) on IgE for FcεRI and FcεRII (29–31). These studies provided only limited data on the sections of IgE involved in binding to its receptors. Studies using protein fragments of IgE were suggestive, but interpretation was complicated by the uncertain retention of native conformation of IgE (32,33). The ability of low-affinity peptides to block formation of the IgE-FcεRI complex was problematic given the high affinity of this interaction ($K_d = 10^{-9}$–10^{-10} M^{-1}) (1,34,35). More recently, both randomized and specific phage-displayed Cε3-Cε4 peptide libraries have been used to screen for IgE epitopes that are bound by a nonanaphylatogenic anti-IgE monoclonal (36). These peptide mimitopes, which may partially mimic the IgE binding site for FcεRI, have been successfully used to generate polyclonal nonanaphylactogenic antisera, which block IgE-mediated mast cell degranulation in vivo (37). Such IgE-like antigens could theoretically be used as vaccine immunogens to generate anti-idiotypic, neutralizing, and nonanaphylactogenic IgE antibodies (38); however, such an approach might not have the same safety profile compared to nonanaphylactogenic monoclonal anti-IgE therapy.

The first significant breakthrough in defining Fc domains involved in FcεRI binding occurred when IgG/IgE chimeric immunoglobulins were constructed and tested for binding to IgE and IgG receptors (39–42). These studies implicated the IgE Cε3 domain as the major, if not sole, binding site on IgE for FcεRI. Once the Cε3 domain was implicated, a more refined mapping of individual

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residues in this domain was undertaken (23). By a combination of IgG loop substitutions in IgE and alanine substitution for all putatively exposed residues in the Cε3 domain, several residues were found to be important for IgE binding to FcεRI: Arg408(376), Ser411(378), Lys415(380), Glu452(414), Arg465(427), and Met469(430) (IgE residues are numbered according to Ref. 43; residue numbers in parentheses are according to Ref. 44). A subsequent study uncovered P364(333) and R365(334) as additional IgE residues involved in binding FcεRI (35). In the crystal structures of IgE, these residues are members of the Cε3 domain N-terminal linker (i.e., the segment connecting the Cε2 and Cε3 domains; Pro364, Arg365), loop CD (i.e., the loop connecting β-strands C and D in domain Cε3; Arg408, Ser411, Lys415), β-strand D (Arg428), loop EF (Glu452), and β-strand F (Arg465, Met469). A role for the Cε3 domain loop AB was proposed (45). However, a later study by the same group contradicted this, showing that loop AB is not directly involved but that a correct conformation of this loop is necessary for proper binding (46); this same conclusion was reached by an earlier study (23).

Each IgE molecule has two identical heavy chains and, hence, could have two identical binding sites per IgE. Three lines of evidence point to a nonequivalence between the two sites. First, IgE forms only a one-to-one complex with FcεRI (47,48). This can be rationalized from a biological perspective—if *both* sites on IgE could bind to FcεRI, then a single IgE molecule could crosslink two FcεRI molecules independent of its being bound to antigen. Second, biophysical measurements of IgE suggest that IgE has a bent configuration both in solution and when bound to FcεRI (48–52). Such a bent structure may result in the two binding sites having different conformation and/or exposure. Third, two antibodies have been reported that bind to IgE free in solution but will not bind to FcεRI-bound IgE (53,54). IgE should have two copies of the epitope of these anti-IgE antibodies, one on each heavy chain. If the two copies of the epitope were equivalent, one might expect that the second site would be available even if the first site were interacting with FcεRI.

Finally, it is apparent that when IgE binds to FcεRI a conformational change occurs, which may involve a change in either or both the IgE and FcεRI. This is supported by hydrodynamic studies of complex formation in solution (48) as well as by studies of anti-IgE antibody binding (55). In the latter study it was determined that, in solution, an anti-IgE antibody could form three types of complexes with IgE depending on the molar ratios of the two components. At a 1:1 molar ratio, a hexamer consisting of three anti-IgE and three IgE was found. When IgE was in excess, a trimer consisting of a 1:2 ratio of anti-IgE:IgE was obtained. When the anti-IgE antibody was in excess, a trimer consisting of 2:1 ratio of anti-IgE:IgE was obtained. These data suggest that when IgE is free in solution, both of its identical epitopes are available to the anti-IgE antibody. In contrast, when IgE is complexed with FcεRI, the anti-IgE does not bind to it;

hence, either both epitopes are masked binding of a single FcεRI or a conformational change occurs, which makes the second epitope unavailable for anti-IgE binding.

Studies on the domains of FcεRI involved in binding to IgE have also been reported. The FcεRI receptor is comprised of three membrane-bound chains, which can, in humans, form a trimeric $\alpha\gamma 2$ or tetrameric $\alpha\beta\gamma 2$ complex (7). The α chain is involved in IgE binding while the β and γ chains are involved in signal transduction. The extracellular portion of the α-chain consists of two immunoglobulin superfamily domains, and prior to solution of the IgE-FcεRIα complex (20), the second membrane proximal domain had been implicated as the major site for interaction with IgE (22,27,28,56,57). Other studies elucidated the specific FcεRI residues involved in binding to IgE (22,24,34). The IgE-FcεRIα crystal structure shows that binding interactions are formed between both Cε3 domains of the IgE-Fc and one side of the second FcεRI immunoglobulin-like domain and at the top of the interface between the FcεRI immunoglobulin-like domains (20). The crystal structure also shows that binding of one FcεRIα to one IgE prevents binding of a second FcεRIα, as previously determined in binding experiments.

II. Nonanaphylactogenic Anti-IgE Antibody Identification and Humanization

Early studies on anti-IgE antibodies using mouse IgE and rat FcεRI-expressing RBL cells and FcεRII expressing B cells focused on elucidating their binding epitopes by competition and cross blocking studies and relating these epitopes to those segments of IgE involved in binding to FcεRI and FcεRII (58–63). These studies also led to the conclusion that one type of therapeutically useful anti-IgE antibody would bind to soluble-free IgE, thereby preventing IgE binding to FcεRI, but would not interact with FcεRI-bound IgE (60,62). Failure of the anti-IgE antibody to bind to FcεRI-bound IgE would eliminate the possibility of anti-IgE crosslinking multiple FcεRI complexes, leading to mast cell degranulation and release of inflammatory mediators, which, in turn, would elicit an unfavorable biological response, such as symptoms associated with allergy or asthma (58,63,64). Two murine antibodies, MaE11 (53,65) and TES-C21 (54), directed against human IgE were identified by screening for monoclonals, which bound to free IgE in solid-phase ELISA assays but failed to bind to IgE bound to native EcεRI, either on human basophils or cell lines expressing recombinant FcεRI α-subunit.

However, as a therapeutic, the murine antibody could not be the molecule of choice because clinical use of nonhuman antibodies has identified three fundamental problems. First, nonhuman antibodies cause a human immune response,

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which can reduce the therapeutic value of the nonhuman antibody (66–71). Second, therapeutic efficacy is reduced by the relatively rapid clearance of the nonhuman antibody compared to human ones (72). Finally, nonhuman antibodies generally show only weak recruitment of effector functions (e.g., antibody-dependent cell-mediated cytolysis), which may be desirable, or essential, for efficacy.

For both of the reported anti-IgE antibodies, the process of humanization was utilized to make them therapeutically acceptable. Humanized antibodies significantly reduce the amount of nonhuman sequence in the molecule and have been shown to reduce the occurrence of anti-antibody response when used clinically (73). This technique, pioneered by Winter and colleagues (74–76), involves transplantation of the nonhuman antigen-binding loops (also referred to as complementarity-determining regions, or CDRs) onto a human antibody framework. In addition to CDRs, select nonhuman framework residues may also be incorporated into the humanized antibody in order to maintain proper CDR conformation (77) or because they interact directly with the antigen (78). The humanized antibody, when properly constructed, contains approximately 5–8% nonhuman residues, the majority of which will be in the CDRs. Several humanized antibodies are now successful therapeutics (79–81), and many more are currently in clinical trials (see Chap. 20).

Three techniques have been used for humanization of antibodies. First, in the ''best-fit'' method, humanized antibodies have been designed by comparing the sequence of the murine antibody of interest to a database of human antibody sequences and choosing the human antibody closest in sequence to that of the murine antibody (54,82,83). Second, in a process referred to as ''resurfacing,'' only the non-CDR, solvent-exposed murine residues are changed to a human sequence; the murine residues buried in the interior of the light (VL) and heavy (VH) chain variable domains and at the VL/VH interface are retained as murine (84,85). Third, a human framework derived from consensus sequences of human VL and VH subgroups is used (as opposed to choosing a human framework closest to the murine antibody sequence) (86). This provides for use of the most common framework found among human IgG antibodies and eliminates possible idiosyncracies present in any individual framework, both of which would reduce the chance of an immunogenic response against the humanized antibody.

The anti-IgE antibody TES-C21 was humanized utilizing both the ''best-fit'' and consensus methods (54). Though the optimal overall binding to IgE was achieved with the humanized antibody designed using the consensus method, no clear advantage of one method over the other was apparent. The resulting humanized antibodies bound to IgE with an affinity similar to the parent murine TES-C21. Of these, the humanized antibody with optimal binding contained nine murine framework residues in VH and four murine framework residues in VL.

A different anti-IgE antibody, E25, was also humanized (53,87) and is currently in clinical trials (88–93). For this humanization, the consensus method

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was used, and 13 variants of the humanized antibody were evaluated to probe the importance of framework residues on antibody binding. In the final humanized antibody, five residues in the human consensus framework were changed to their murine counterpart (i.e., from the parent murine antibody) to provide for binding comparable to that of the original murine antibody. Both the parent murine and humanized antibodies blocked binding of human IgE to CHO 3D10 cells expressing human FcεRI α chain (1) but did not bind to FcεRI-bound IgE (53).

In addition to the humanization, several amino acids in the CDRs important for binding of the antibody to IgE were elucidated (53). The sequence of the E25 CDRs includes several charged side chains (Fig. 1). CDR-L1 contains three Asp residues, while CDR-L3 possesses His, Glu, and Asp; in CDR-H3 there are three His residues. A computer model of humanized E25 pointed to the spatial proximity of all of these charged residues (Fig. 2). In contrast, the lone Asp H54 in CDR-H2 is spatially separated from the other charged residues. E25 variants were generated in which one or more charged residues were replaced with Ala. In CDR-L1 alteration of one of the three Asp residues, Asp L32, to Ala effectively abolished binding to IgE, while the other two had minimal effect. Simultaneous alteration of GluL93Ala and AspL94Ala in CDR-L3 also reduced binding, though much less than for AspL32Ala. Individually substituting the three His residues in CDR-H3 with Ala resulted in either slightly improved binding or a threefold reduction in binding. However, simultaneous alteration of all three His residues abolished binding. While these substitutions cannot discern whether these charged residues are involved in direct binding to IgE or provide some conformational stability to their respective CDRs, they show that CDR-L1 and CDR-H3 are important determinants in IgE binding for the E25 antibody. The concentration of charges in the CDRs, especially in CDR-L1 and CDR-H3, suggested that the E25 epitope on IgE also had a concentration of charged residues, which may have important implications for developing future IgE antagonists.

The E25 antibody was chosen for humanization because it fulfilled the criteria of binding to free IgE but not FcεRI-bound IgE. This suggested that the E25 epitope on IgE might overlap the binding site on IgE for FcεRI. The panel of IgE variants used to elucidate the FcεRI-binding site on IgE (23) were also used to determine the E25 epitope. Table 1 shows that the IgE residues involved in binding to the E25 antibody are a subset of those involved in FcεRI binding. The importance of the binding site overlap between the E25 epitope on IgE and that of FcεRI is illustrated by the mapped epitopes of two other anti-IgE antibodies. MaE13 and MaE17 (65) were also identified in the initial screen resulting in MaE11. However, in vitro cell-based assays measuring histamine release showed that MAE13 allowed 5% histamine release, MaE17 allowed 10% histamine release, while MaE11 exhibited no histamine release (one of the criteria leading to the choice of MaE11 as the parent antibody of humanized E25 as therapeutic antibody). Table 1 shows that the MaE13 and MaE17 epitopes overlap

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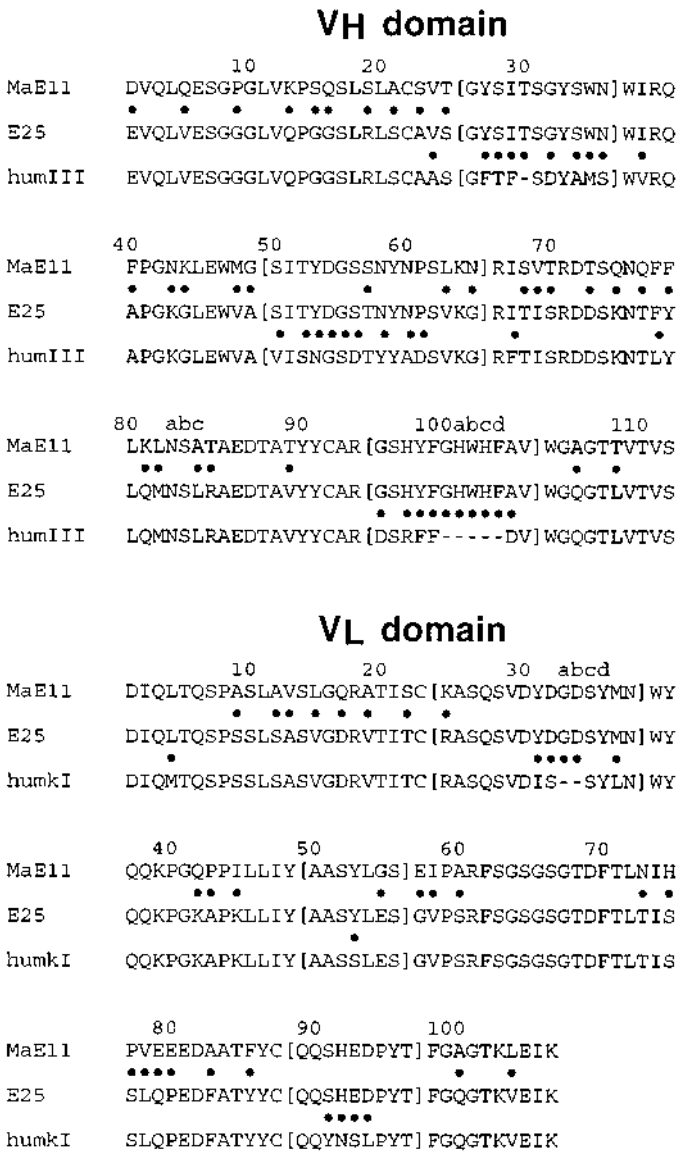

Figure 1 Alignment of amino acid sequences of murine MaE11, humanized E25, and human consensus sequences. Differences between sequences are noted by bullets.

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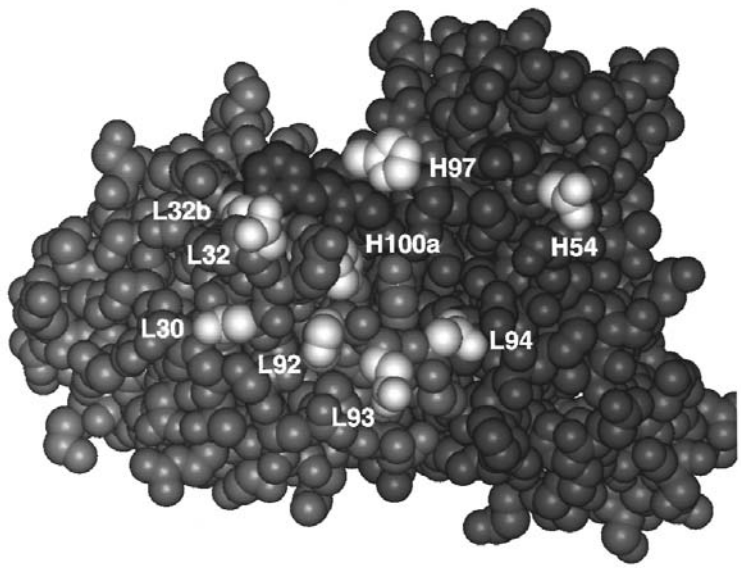

Figure 2 Model of E25 Fab: VH residues are colored dark gray, VL residues are light gray, charged residues are colored white and labeled (L = light chain, H = heavy chain).

that of MaE11 (and E25) but are slightly displaced compared to that of MaE11 and FcεRI. The differences in the epitopes of the three antibodies in conjunction with the histamine release data suggest that in order to be therapeutically efficacious (i.e., zero tolerance for histamine release), the antibody must bind to and efficiently block the same site on IgE as that utilized by FcεRI.

When the humanized E25 antibody was being characterized for use in clinical trials, it was discovered that an Asp-Gly sequence in CDR-L1 could undergo a rearrangement to form isoaspartic acid (94). This sequence was in the parent murine antibody CDR-L1 and hence was included in the humanized antibody. Formation of isoaspartic acid in CDR-L1 abrogated binding of the humanized antibody, reducing binding of the Fab form to IgE by 87%. Though the low level of isomerization did not prevent use of E25 in clinical trials, further studies on E25 were undertaken to determine if the problem could be overcome. Changing the CDR-L1 AspL32 to Ala or Glu prevented isomerization, but these changes also reduced binding of the E25 antibody to IgE by 73% and 67%, respectively. From the study on the residues in the E25 CDRs important for binding to IgE (53), it was found that changing certain CDR residues to Ala improved binding, namely Asp H54 and His H105. Simultaneously changing AspH54Ala and HisH105Ala in conjunction with AspL32Ala (the problematic Asp) resulted in an

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Table 1 Epitope Mapping of Human FcεRI and Anti-IgE mAbs[a]

Residue number[b]	Human Fcε3	Changed to	FcεRI	MaE11	E25	MaE13	MaE17
Loop BC							
398–401	SKGT	AAGA	+++[c]	+++		+++	++
β-Strand	C						
403, 405	NLT	ALA	+++	+++	+++	+++	+++
Loop CD							
407	S	E	+++	+	−	+++	+++
407	S	Q	+++	++	−	+++	+++
408	R	E	−	+	+	+	++
411	S	Q	+	+	+	+	++
415	K	D	++	++	−	+++	+++
418	N	A	+++	+++	+++	+++	+++
Loop EF							
450	E	A	+++	+++	+++	+++	+++
452	E	R	−	−	−	−	+
452	E	Q	−	−	−	−	+
453	T	R	+++	+++		++	+++
β-Strand	F						
455–459	QCRVT	ACAVA	+++	−	−	++	+++
Loop FG							
465	R	E	+	++	−	+++	+++
469	M	A	++	−	−	−	+++
β-Strand	G						
471, 473	STT	ATA	+++	+++	+++	+++	+++

[a] human IgE residues comprising epitope of MaE antibodies and FcεRI:

FcεRI		R408	S411	K415	E452		R465	M469	
MaE11	S407	R408	S411	K415	E452		R465	M469	β-strandF
MaE13		R408	S411		E452	T453		M469	
MaE17		R408	S411		E452				LoopBC

[b] Human IgE residue numbers according to Ref. 43.

[c] +++, 100–75% compared to native human IgE; ++, 74–45%; +, 44–15%; −, 14–0%.

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E25 variant with binding reduced only twofold compared to that of the original E25.

III. Possible Mechanisms of Action of Anti-IgE E25 Antibody

When the co-crystal structure of IgE/FcεRIα was determined, only a few of the IgE residues previously mapped by mutagenesis as part of the FcεRI-binding site on IgE were found to contact FcεRI (20), e.g., Pro364, Arg365, and Arg465. Many others, e.g., Arg408, Ser411, Lys415, Arg428, Glu452, and Met469, were not part of the IgE/FcεRI interface. This discrepancy might be due to some of the mutations in IgE effecting a conformational change in IgE domain Cε3, which abrogated binding to FcεRI, even though the altered amino acids in IgE did not directly interact with FcεRI.

The epitope of the anti-IgE murine MaE11 and its humanized counterpart E25 mapped to IgE residues which, for the most part, are not part of the IgE/FcεRI interface (Table 1); these include Ser407, Arg408, Ser411, Lys415, Glu452, and Met469. As noted for FcεRI, mutations at some of these residues may have effected a conformational change in the IgE (e.g., Arg408, Ser411, Glu452). If we exclude these three residues, the E25 epitope on IgE would consist of Ser407, Lys415, Met469, and β-strand D. Surprisingly, this epitope is distant from the IgE/FcεRI interface in the co-crystal structure (20), and it is uncertain how E25 would exert its effect by sterically hindering binding of IgE to FcεRI. In contrast, the epitope of MaE17 includes IgE loop BC and seems closer to the FcεRI-binding site found in the crystal structure than the epitope of E25 (Table 1). However, MaE17 allowed 10% histamine release, whereas MaE11 allowed no histamine release, suggesting that steric hindrance alone may not fully explain the mechanism of E25.

Another possibility is that the E25 exerts its influence by locking the IgE into a conformation that precludes FcεRI binding. The latter is suggested by comparison of the crystal structures of free IgE-Fc and IgE bound to FcεRIα (20,21). Comparison of these structures shows a conformational change in IgE when bound to FcεRI, with an ''open'' form in free IgE and a ''closed'' form in bound IgE. There are relatively large shifts between the two forms in the position of several loops, including loops CD and EF (which comprise some of the E25 epitope on IgE). It is feasible that when E25 binds to free IgE it locks the IgE in the ''closed'' form, thereby preventing FcεRI binding (which requires the ''open'' form).

IV. Summary

The current results with an anti-IgE monoclonal antibody in clinical trials (88–93) have provided proof that preventing binding of IgE to its high-affinity recep-

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tor, FcεRI, can indeed mitigate the symptoms of asthma and possibly also allergic rhinitis. The recent determination of the crystal structures of the IgE, FcεRIα and IgE-FcεRIα (19–21), in conjunction with mapping of the FcεRI-binding site on IgE (23,35), has laid the groundwork for the next generation of antiasthma and antiallergy therapeutics. The structural data will, hopefully, aid in the design of small, orally available therapeutics that can *prevent* the release of inflammatory mediators and cytokines, as opposed to current therapeutics that act *after* the release of these mediators.

References

1. Hakimi J, Seals C, Kondas JA, Pettine L, Danho W, Kochan J. The α subunit of the human IgE receptor (FcERI) is sufficient for high affinity IgE binding. J Biol Chem 1990; 265:22079–22081.
2. Ishizaka K, Ishizaka T, Lee EH. Biologic function of the Fc fragments of E myeloma protein. Immunochemistry 1970; 7:687–702.
3. Metzger H, Alcaraz G, Hohman R, Kinet J-P, Pribluda V, Quarto R. The receptor with high affinity for immunoglobulin E. Ann Rev Immunol 1986; 4:419–470.
4. Bieber T, de la Salle H, Wollenberg A, Hakimi J, Chizzonitte R, Ring J, Hanau D, de la Salle C. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (FcεRI). J Exp Med 1992; 175:1285–1290.
5. Wang B, Rieger A, Kilgus O, Ochial K, Maurer D, Fodinger D, Kinet J-P, Stingl G. Epidermal Langerhans cells from normal human skin bind monomeric IgE via Fc epsilon RI. J Exp Med 1992; 175:1353–1365.
6. Maurer D, Fiebiger E, Reininger B, Wolff-Winiski B, Jouvin MH, Kilgus O, Kinet J-P, Stingl G. Expression of functional high affinity immunoglobulin E receptors (Fc epsilon RI) on monocytes of atopic individuals. J Exp Med 1994; 179:745–750.
7. Kinet J-P. The high-affinity IgE Receptor (FcεRI: from physiology to pathology). Ann Rev Immunol 1999; 17:931–972.
8. Shiue L, Green J, Green OM, Karas JL, Morgenstern JP, Ram MK, Taylor MK, Zoller MJ, Zydowsky LD, Bolen JB, et al. Interaction of p72syk with the gamma and beta subunits of the high affinity receptor for immunoglobulin E, Fc epsilon RI. Mol Cell Biol 1995; 15:272–281.
9. Lin S, Cicala C, Scharenberg AM, Kinet J-P. The FcεRI β subunit functions as an amplifier of FcεRIγ-mediated cell activation signals. Cell 1996; 85:985–995.
10. Dombrowicz D, Lin S, Flamand V, Brini AT, Koller BH, Kinet J-P. Allergy-associated FcR beta is a molecular amplifier of IgE and IgG mediated in vivo responses. Immunity 1998; 8:517–529.
11. Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of FcεRI or to calcium ionophores. Nature 1989; 339:64–67.
12. Austen KF, Wasserman SI, Goetzel EJ. Mast cell-derived mediators: structural and functional diversity and regulation of expression. In: S. Johanson SGO, Strandeng K, Uvnas B, eds. Molecular Aspects of the Acute Allergic Reactions. New York: Plenum Press, 1976:293.

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13. Ishizaka T, Conrad DH, Schulman ES, Sterk AR, Ishizaka K. Biochemical analysis of initial triggering events of IgE-mediated histamine release from human lung mast cells. J Immunol 1983; 130:2357–2362.
14. Sutton BJ, Gould HJ. The human IgE network. Nature 1993; 366:421–428.
15. Delespesse G, Sarfati M, Wu CY, Fournier S, Letellier M. The low-affinity receptor for IgE. Immunol Rev 1992; 125:77–97.
16. Mossalayi MD, Arock M, Debre P. CD23/FcεRII: signaling and clinical implication. Int Rev Immunol 1997; 16:129–146.
17. Bonnefoy JY, Lecoanet-Henchoz S, Gauchat JF, Graber P, Aubry JP, Jeannin P, Plater-Zyberk C. Structure and functions of CD23. Intl Rev Immunol 1997; 16:113–128.
18. Dembo M, Goldstein B, Sobotka AK, Lichtenstein LM. Degranulation of human basophils: quantitative analysis of histamine release and desensitization due to bivalent penicilloyl hapten. J Immunol 1979; 123:1864–1872.
19. Garman S, Kinet J-P, Jardetsky TS. Crystal structure of the human high-affinity IgE receptor. Cell 1998; 95:951–961.
20. Garman SC, Wurzburg BA, Tarchevskaya SS, Kinet J-P, Jardetzky TS. Structure of the Fc fragment of human IgE bound to its high-affinity receptor FcεRIα. Nature 2000; 406:259–266.
21. Wurzburg BA, Garman SC, Jardetzky TS. Structure of the human IgE-Fc Cε3-Cε4 reveals conformational flexibility in the antibody effector domains. Immunity 2000; 13:379–385.
22. Mallamaci MA, Chizzonite R, Griffin M, Nettleton M, Hakimi J, Tsien WH, Kochan JP. Identification of sites on the human FcεRIα subunit which are involved in binding human and rat IgE. J Biol Chem 1993; 268:22076–22083.
23. Presta L, Shields R, O'Connell L, Lahr S, Porter J, Gorman C, Jardieu P. The binding site on human immunoglobulin E for its high affinity receptor. J Biol Chem 1994; 42:26368–26373.
24. Hulett MD, Brinkworth RI, C. McKenzie IF, Hogarth PM. Fine structure analysis of interaction of FcεRI with IgE. J Biol Chem 1999; 274:13345–13352.
25. McDonnell JM, Beavil AJ, Mackay GA, Jameson BA, Korngold R, Gould HJ, Sutton BJ. Structure based design and characterization of peptides that inhibit IgE binding to its high-affinity receptor. Nature Struct Biol 1996; 3:419–425.
26. McDonnell JM, Beavil AJ, Mackay GA, Henry AJ, Cook JPD, Gould HJ, Sutton BJ. Structure-based design of peptides that inhibit IgE binding to its high-affinity receptor FcεRI. Biochem Soc Trans 1997; 25:387–392.
27. Robertson MW. Phage and *Escherichia coli* expression of the human high affinity immunoglobulin E receptor α-subunit ectodomain. J Biol Chem 1993; 268:12736–12743.
28. Scarselli E, Esposito G, Traboni C. Receptor phage. Display of functional domains of the human high affinity IgE receptor on the M13 phage surface. FEBS Lett 1993; 329:223–226.
29. Stanworth DR. Use of synthetic peptides representative of Fc sequences in the delineation of IgG and IgE effector functions. In: Peeters H, ed. Protides of Biological Fluids. New York: Pergamon Press, 1986:169–172.
30. Burt DS, Stanworth DR. Inhibition of rat IgE to rat mast cells by synthetic IgE peptides. Eur J Immunol 1987; 17:437–440.

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31. Nio N, Seguro K, Ariyoshi Y, Nakanishi K, Kita A, Ishii K, Nakamura H. Inhibition of histamine release by synthetic human IgE peptide fragments: structure-activity studies. In: Yanaihara N, ed. Peptide Chemistry. Osaka: Protein Research Foundation, 1990:203–208.
32. Helm B, Marsh P, Vercelli D, Padlan E, Gould H, Geha R. The mast cell binding site on human immunoglobulin E. Nature 1988; 331:180–183.
33. Basu M, Hakimi J, Dharm E, Kondas JA, Tsien W-H, Pilson RS, Lin P, Gilfillan A, Haring P, Braswell EH, Nettleton MY, Kochan JP. Purification and characterization of human recombinant IgE-Fc fragments that bind to the human high affinity IgE receptor. J Biol Chem 1993; 268:13118–13127.
34. Cook JPD, Henry AJ, McDonnell JM, Owens RJ, Sutton BJ, Gould HJ. Identification of contact residues in the IgE binding site of human FcεRIα. Biochemistry 1997; 36:15579–15588.
35. Henry AJ, Cook JPD, McDonnell JM, Mackay GA, Shi J, Sutton BJ, Gould HJ. Participation of the N-terminal region of Cε3 in the binding of human IgE to its high-affinity receptor FcεRI. Biochemistry 1997; 36:15568–15578.
36. Rudolf MP, Vogel M, Kricek F, Ruf C, Zurcher AW, Reushcel R, Auer M, Miescher S, Stadler B. Epitope-specific antibody response to IgE by mimotope immunization. J Immunol 1998; 160:3315–3321.
37. Rudolf MP, Furukawa K, Miescher S, Vogel M, Kricek F, Stadler B. Effect of anti-IgE antibodies on FcεRI-bound IgE. J Immunol 1996; 157:5646–5652.
38. Stadler BM, Zucher AW, Miescher S, Kricek F, Vogel M. Mimotope and anti-idotypic vaccines to induce an anti-IgE response. Int Arch Allergy Immunol 1999; 118:119–121.
39. Weetall M, Shopes B, Holowka D, Baird B. Mapping the site of interaction between murine IgE and its high affinity receptor with chimeric Ig. J Immunol 1990; 145: 3849–3854.
40. Nissim A, Jouvin M-H, Eshhar Z. Mapping the high affinity Fcε receptor binding site to the third constant region domain of IgE. EMBO J 1991; 10:101–107.
41. Keegan AD, Fratazzi C, Shopes B, Baird B, Conrad DH. Characterization of new rat anti-mouse IgE monoclonals and their use along with chimeric IgE to further define the site that interacts with FcεRII and FcεRI. Mol Immunol 1991; 28:1149–1154.
42. Nissim A, Schwarzbaum S, Siraganian R, Esshar Z. Fine specificity of the IgE interaction with the low and high affinity Fc receptor. J Immunol 1993; 150:1365–1374.
43. Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C. Sequences of Proteins of Immunological Interest, 5th ed. Washington, DC: Public Health Service, NIH, 1991.
44. Dorrington KJ, Bennich HH. In: Bach MK, ed. Immediate Hypersensitivity: Modern Concepts and Developments. New York: Marcel Dekker, 1978:47–71.
45. Helm BA, Sayers I, Higginbottom A, Cantarelli-Machado D, Ling Y, Ahmad K, Padlan EA, Wilson APM. Identification of the high affinity receptor binding region in human immunoglobulin E. J Biol Chem. 1996; 271:7494–7508.
46. Sayers I, Cain SA, Swan JRM, Pickett MA, Watt PJ, Holgate ST, Padlan EA, Schuck P, Helm BA. Amino acid residues that influence FcεRI-mediated effector functions of human immunoglobulin E. Biochemistry 1998; 37:16152–16164.
47. Kanellopoulos JM, Liu TY, Poy G, Metzger H. Composition and subunit structure of the cell receptor for immunoglobulin E. J Biol Chem 1980; 225:9060–9066.

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48. Keown MB, Ghirlando R, Young RJ, Beavil AJ, Owens RJ, Perkins SJ, Sutton BJ, Gould HJ. Hydrodynamic studies of a complex between the Fc fragment of human IgE and a soluble fragment of the FcεRI α chain. Proc Natl Acad Sci USA 1995; 92:1841–1845.
49. Davis KG, Glennie M, Harding SE, Burton DR. A model for the solution conformation of rat IgE. Biochem Soc Trans 1990; 18:935–936.
50. Holowka D, Wensel T, Baird B. A nanosecond fluorescence depolarization study on the segmental flexibility of receptor-bound immunoglobulin E. Biochemistry 1990; 29:4607–4612.
51. Zheng Y, Shopes B, Holowka D, Baird B. Dynamic conformations compared for IgE and IgG1 in solution amd bound to receptors. Biochemistry 1992; 31:7446–7436.
52. Baird B, Zheng Y, Holowka D. Structural mapping of IgE-FcεRI, an immunoreceptor complex. Acc Chem Res 1993; 26:428–434.
53. Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, Jardieu PM. Humanization of an antibody directed against IgE. J Immunol 1993; 151:2623–2632.
54. Kolbinger F, Saldanha J, Hardman N, Bendig MM. Humanization of a mouse anti-human IgE antibody: a potential therapeutic for IgE-mediated allergies. Prot Eng 1993; 6:971–980.
55. Liu J, Lester P, Builder S, Shire SJ. Characterization of complex formation by humanized anti-IgE monoclonal antibody and monoclonal human IgE. Biochemistry 1995; 34:10474–10482.
56. Hogarth PM, Hulett MD, Ierino FL, Tate B, Powell MS, McKenzie IFC, Brinkworth RI. Identification of the immunoglobulin binding regions (IBR) of Fc gamma RII and Fc epsilon RI. Immunol Rev 1992; 125:21–35.
57. Hulett MD, McKenzie IFC, Hogarth PM. (1993) Chimeric Fc receptors identify immunoglobulin-binding regions in human FcγRII and FcεRI. Eur J Immunol 1993; 23:640–645.
58. Burt DS, Hastings GZ, Stanworth DR. Localisation of binding site(s) on IgE for specific receptor on mast cells. In: Peeters H, ed. Protides of Biological Fluids. New York: Pergamon Press, 1986:243–246.
59. Baniyash M, Esshar Z, Rivnay B. Relationships between epitopes on IgE recognized by defined monoclonal antibodies and by the Fcε receptor on basophils. J Immunol 1986; 136:588–593.
60. Baniyash M, Kehry M, Esshar Z. Anti-IgE monoclonal antibodies directed at the Fcε receptor binding site. Mol Immunol 1988; 25:705–711.
61. Robertson MW, Liu F-T. IgE structure-function relationships defined by sequence directed antibodies induced by synthetic peptides. Mol Immunol 1988; 25:103–113.
62. Del Prado JM, Jimeno L, Obispo T, Carreira J. Monoclonal antibodies against human IgE. Identification of an epitope sharing properties with the high-affinity receptor binding site. Mol Immunol 1991; 28:839–844.
63. Hook WA, Zinsser FU, Berenstein EH, Siraganian RP. Monoclonal antibodies defining epitopes on human IgE. Mol Immunol 1991; 28:631–639.
64. Stadler BM, Gang Q, Vogel M, Jarolim E, Miescher S, Aebischer I, de Weck AL. IgG anti-IgE autoantibodies in immunoregulation. Int Arch Allergy Appl Immunol 1991; 94:83–86.

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65. Shields RL, Werther WR, Zioncheck K, O'Connell L, Klassen T, Fendly B, Presta LG, Jardieu PM. Anti-IgE monoclonal antibodies that inhibit allergen-specific histamine release. Int Arch Allergy Immunol 1995; 107:412–413.
66. Miller RA, Oseroff AR, Stratte PT, Levy R. Monoclonal antibody therapeutic trials in seven patients with T-cell lymphoma. Blood 1983; 62:988–995.
67. Sears HF, Herlyn D, Steplewski Z, Koprowski H. Effects of monoclonal antibody immunotherapy on patients with gastrointestinal adenocarcinoma. J Biol Response Mod 1984; 3:138–150.
68. Shawler DL, Bartholomew RM, Smith LM, Dillman RO. Human immune response to multiple injections of murine monoclonal IgG. J Immunol 1985; 135:1530–1535.
69. Jaffers GJ, Fuller TC, Cosimi AB, Russell PS, Winn HJ, Colvin RB. Monoclonal antibody therapy. Anti-idiotypic and non-anti-idiotypic antibodies to OKT3 arising despite intense immunosuppression. Transplantation 1986; 41:572–578.
70. Khazaeli MB, Saleh MN, Wheeler RH, Huster WJ, Holden H, Carrano R, LoBuglio AF. Phase I trial of multiple large doses of murine monoclonal antibody CO17-1A. II. Pharmacokinetics and immune response. J Natl Cancer Inst 1988; 80:937–942.
71. Brüggemann M, Winter G, Waldmann H, Neuberger MS. The immunogenicity of chimeric antibodies. J Exp Med 1989; 170:2153–2157.
72. Hakimi J, Chizzonite R, Luke DR, Familletti PC, Bailon P, Kondas JA, Pilson RS, Lin P, Weber DV, Spence C, Mondini LJ, Tsien W, Levin JL, Gallati VH, Korn L, Waldmann TA, Queen C, Benjamin WR. Reduced immunogenicity and improved pharmacokinetics of humanized anti-Tac in cynomolgus monkeys. J Immunol 1991; 147:1352–1359.
73. Vaughan TJ, Osbourn JK, Tempest PR. Human antibodies by design. Nature Biotechnol 1998; 16:535–539.
74. Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 1986; 321:522–525.
75. Verhoeyen M, Milstein C, Winter G. Reshaping human antibodies: grafting an antilysozyme activity. Science 1988; 239:1534–1536.
76. Reichmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. Nature 1988; 332:323–327.
77. Chothia C, Lesk AM, Tramontano A, Levitt M, Smith-Gill SJ, Air G, Sheriff S, Padlan EA, Davies D, Tulip WR, Colman PM, Spinelli S, Alzari PM, Poljak RJ. Conformations of immunoglobulin hypervariable regions. Nature 1989; 342:877–883.
78. Amit AG, Mariuzza RA, Phillips SEV, Poljak RJ. Three-dimensional structure of an antigen-antibody complex at 2.8 Å resolution. Science 1986; 233:747–753.
79. Goldenberg MM. Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer. Clin Therapeutics 1998; 21:309–318.
80. Meissner HC, Welliver RC, Chartrand SA, Law BJ, Weisman LE, Dorkin HL, Rodriguez WJ. Immunoprophylaxis with palivizumab, a humanized respiratory syncytial virus monoclonal antibody, for prevention of respiratory syncytial virus infection in high risk infants: a consensus opinion. Pediatr Infect Dis J 1999; 18:223–231.
81. Ekberg H, Backman L, Tufveson G, Tyden G. Zenapax (daclizumab) reduces the

MARCEL DEKKER, INC.
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incidence of acute rejection episodes and improves patient survival following renal transplantation. No 14874 and No 14393 Zenapax Study Groups. Transplant Proc 1999; 31:267–268.
82. Co MS, Deschamps M, Whitley RJ, Queen C. Humanized antibodies for antiviral therapy. Proc Natl Acad Sci USA 1991; 88:2869–2873.
83. Kettleborough CA, Saldanha J, Heath VJ, Morrison CJ, Bendig MM. Humanization of a mouse monoclonal antibody by CDR-grafting: the importance of framework residues on loop conformation. Prot Eng 1991; 4:773–783.
84. Padlan EA. A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties. Mol Immunol 1991; 28:489–498.
85. Roguska MA, Pedersen JT, Keddy CA, Henry AH, Searle SJ, Lambert JM, Goldmacher VS, Blattler WA, Rees AR, Guild BC. Humanization of murine monoclonal antibodies through variable domain resurfacing. Proc Natl Acad Sci USA 1994; 91: 969–973.
86. Carter P, Presta L, Gorman CM, Ridgway JBB, Henner D, Wong WLT, Rowland AM, Kotts C, Carver ME, Shepard MH. Humanization of an anti-p185^{HER2} antibody for human cancer therapy. Proc Natl Acad Sci USA 1992; 89:4285–4289.
87. Shields RL, Werther WR, Zioncheck K, O'Connell L, Fendly B, Presta LG, Thomas D, Saban R, Jardieu PM. Inhibition of allergic reactions with antibodies to IgE. Int Arch Allergy Immunol 1995; 107:308–312.
88. Boulet LP, Chapman KR, Cote J, Kaira S, Bhagat R, Swystun VA, Laviolette M, Cleland LD, Deschesnes F, Su JQ, DeVault A, Ficl Jr., RB, Cockcroft DW. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. Am J Respir Crit Care Med 1997; 155:1835–1840.
89. Casale TB, Bernstein IL, Busse WW, LaForce CF, Tinkelman DG, Stoltz RR, Dockhorn RJ, Reimann J, Su JQ, Fick Jr., RB, Adelman DC. Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. J Allergy Clin Immunol 1997; 100:110–121.
90. Demoly P, Bousquet J. Anti-IgE therapy for asthma. Am J Respir Crit Care Med 1997; 155:1825–1827 [see also Am J Respir Crit Care Med 156:1707 for published erratum].
91. Fahy JV, Flemming HE, Wong HH, Liu JT, Su JQ, Reimann J, Fick Jr., RB, Boushey HA. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med 1997; 155:1828–1834.
92. Frew J. Effects of anti-IgE in asthmatic subjects. Thorax 1998; 53:S52–S57.
93. Saini SS, MacGlashan Jr., DW, Sterbinsky SA, Togias A, Adelman DC, Lichtenstein LM, Bochner BS. Down-regulation of human basophil IgE and FcεRIα surface densities and mediator release by anti-IgE infusions is reversible in vitro and in vivo. J Immunol 1999; 162:5624–5630.
94. Cacia J, Keck R, Presta LG, Frenz J. Isomerization of an aspartic acid residue in the complementarity-determining regions of a recombinant antibody to human IgE: identification and effect on binding affinity. Biochemistry 1996; 35:1897–1903.

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4

Mediator Release from Basophils and Mast Cells and Its Relationship to FcεRI Expression and IgE-Suppressing Therapies

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I. Introduction

It has long been recognized that IgE-mediated secretion from tissue mast cells or circulating basophils is a central pathophysiological mechanism responsible for the signs and symptoms of allergic diseases. It has also been recognized that elimination of IgE antibody from the circulation should ameliorate the pathophysiological process associated with atopy. Of course, not all aspects of allergic diseases have been shown to be fully dependent on IgE-mediated function in mast cells and basophils, but even in the case of asthma, where a wide variety of pathophysiological mechanisms probably play a role in the expression of the disease, eliminating the IgE-mediated component should have marked effects on the symptoms and severity of the disease. The trick has been developing a therapeutic that reduces circulating IgE and then providing enough of the agent to chronically and markedly influence the functional responses of IgE-bearing cells. This is not an easy task because these cells are exquisitely sensitive to stimulation. A great deal of knowledge about basophil and mast cell function can be used to make predictions about the requirements for new IgE-suppressing therapeutics. Such therapeutics, if actually demonstrated to suppress circulating IgE and associated cellular responses, could be useful tools for dissecting out the relative roles

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of this arm of the immune response in a variety of atopic diseases, including asthma. A variety of schemes have been proposed to induce a decrease in circulating IgE; one currently undergoing investigation is monoclonal anti-IgE antibody. Coupled with recent knowledge about basophil and mast cell function, studies using anti-IgE antibody in vivo have provided useful insights into the nature of allergic reactions, the cell biology of the high-affinity receptor for IgE (FcεRI), and their relationship to studies of basophil and mast cell function.

II. Secretory Functions of Basophils and Mast Cells

A. Mediator Classes: Histamine and Other Preformed Mediators

Of the inflammatory mediators found in basophils and mast cells, none has received more attention than histamine. This focus is rightfully so given the fact that this mediator is not synthesized to an appreciable quantity by any other cell type in the body, making it a specific marker for basophil and mast cell activation. Under normal conditions, basophils are the predominant source of histamine found in blood. However, following experimental or natural exposure to allergen, these cells will migrate into tissue sites in the lung, nose, and skin where they are known to contribute to the ongoing inflammation by releasing histamine and other pro-inflammatory mediators in what is known as the late phase response (LPR) (1–3). In contrast, it is well accepted, but not fully understood, that mast cell precursors circulate in blood only to ''home in'' on specific organs, where they subsequently develop into mature mast cells and become the predominant source of histamine in these tissue sites. Both cell types synthesize histamine from L-histidine through the activity of histidine decarboxylase and store it in cytoplasmic granules, forming a complex with highly charged proteoglycans (4,5). In basophils this is achieved primarily with chrondroitin sulfate, whereas in mast cells heparin is the predominate proteoglycan. During the latter stages of maturation from precursor cells, both cell types appear to depend on the modulatory actions of hematapoietic growth factors for the upregulation of histamine content. For basophils, both IL-3 and GM-CSF have an important role in this process (6,7), whereas stem cell factor (SCF) has been shown to be critical for human mast cell development in vitro (8–11). Furthermore, the administration of recombinant SCF into humans induces mast cell hyperplasia and activation along with hyperpigmentation of the skin (12). Other cytokines and factors have been shown in vitro to synergize with SCF to promote the maturation of mast cells including IL-6 and prostaglandin E_2 (PGE2) (8), IL-4 (13), and thrombopoietin (14). Human mast cells isolated from a variety of tissues, including lung, skin, or small intestine, have been shown to average 3–8 pg of histamine per cell. In contrast, basophils contain considerably less histamine, averaging approximately 1 pg per cell (15). Interestingly, this amount per basophil is remarkably

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consistent among donor populations. Histamine is released from activated cells following intracellular signals, culminating with the fusion of cytoplasmic granules with the cell membrane and extrusion of their contents extracellularly in a form of exocytosis that has been well characterized ultrastructurally (5). As an inflammatory mediator, histamine is a potent smooth muscle spasmogen and can cause vascular leakage by dilating terminal arterioles and constricting postcapillary venules. In addition to these proinflammatory effects, histamine has been shown to downregulate specific immune responses, particular cytotoxic T-lymphocyte (CTL) responses, by increasing cyclic adenosine monophosphate (cAMP) levels via its binding to H2 receptors on CD8+ T cells (16,17). These inhibitory effects are reversed with H2 receptor antagonists (18,19). In light of this information, it seems possible that histamine may also selectively target and downregulate T-helper (Th)1–like responses with the increasing evidence that basophils promote Th2-like responses by producing IL-4 and IL-13 (see Fig. 1 and below). With respect to this belief, histamine has been shown more recently

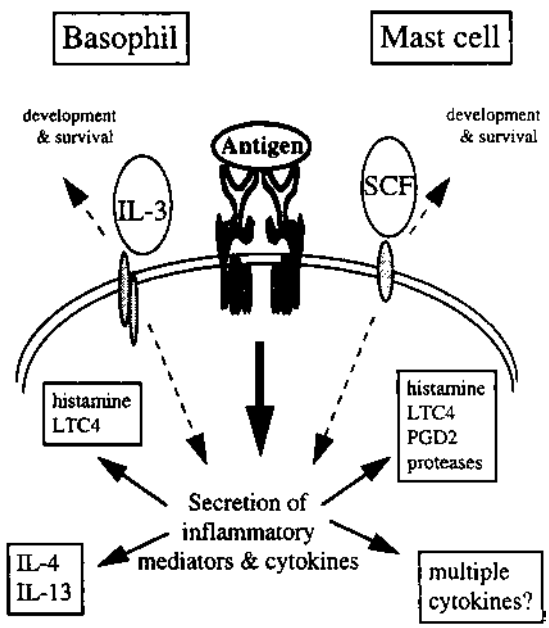

Figure 1 Diagrammatic representation of the secretogogues released from human basophils and mast cells following IgE/FcεRI crosslinking. The binding of specific antigen to receptor-bound IgE initiates signals that culminate in the release of preformed proinflammatory mediators and the synthesis of immunoregulatory cytokines. IL-3 and SCF enhance these secretory events in basophils and mast cells, respectively.

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to suppress IL-12 and IFN-γ production by monocytes and Th1 cell clones, respectively, while promoting the secretion of the Th2 cytokine, IL-10 (20,21).

Mast cells also store neutral proteases within their cytoplasmic granules. At least four molecular forms of tryptase (I, IIβ, III, and α) have been described and are found at high levels within mast cells isolated from most tissues (22,23). Human basophils isolated from normal blood have also been reported to express tryptase, but at levels (0.05 pg/mL) nearly 50–100 times less than those found in mast cells (24) and primarily of the α form (25). As a result, tryptase is commonly used as a specific marker for identifying mast cell involvement in allergic lesions and is typically detected using monoclonal antibodies suitable for immunohistochemistry. Nonetheless, more recent evidence has challenged this belief by showing that tryptase is readily detected in basophils found in the blood of some allergic patients (26), suggesting that not all tryptase-positive cells found in allergic lesions are mast cells, but may also include basophils. Likewise, chymase is another protease that is typically found in mast cells and not basophils. However, not all tryptase-positive mast cells co-stain for chymase, a finding that is very dependent on anatomical location and which has led to the concept of mast cell heterogeneity. The exact role for these proteases in vivo remains highly speculative.

B. Mediator Classes: Lipid Mediators

Basophils and mast cells, within minutes following activation, also generate and release lipid mediators that have potent inflammatory properties. Both PGD2 and leukotriene C_4 (LTC4) are synthesized by mast cells, indicating that cyclo-oxygenase and lipoxygenase enzymes are active in these cells. In contrast, basophils appear to make only LTC4. In fact, the apparent absence of cyclo-oxygenase activity in basophils, combined with evidence showing that prostaglandins are not detected in lavage fluids taken during the LPR, has suggested that the histamine and LTC4 measured in these reactions are derived from basophils accumulating at the lesion site (27). The arachidonic acid serving as the substrate for the synthesis of PGE2 and LTC4 is likely derived from the metabolism of phosphatidylcholine and phosphotidylinositol through the enzymatic activity of phospholipase A_2, or by the sequential action of phospholipase C and diglyceride lipase. The amount of LTC4 per basophil or mast cell (10–100 fg) is far less than their pg/cell levels of histamine (15). However, on a molar basis, LTC4 is up to 6000 times more potent in contracting smooth muscle. Furthermore, LTC4 is metabolized to LTD4 and LTE4 with the combined effect of all three leukotrienes, formerly known as slow-releasing substance of anaphylaxis (SRS-A), having a profound bronchoconstrictive and mucus-producing consequence when released in the respiratory system or inducing a prolonged wheal-and-flare reaction in the skin. These mediators, in fact, are thought to be major contributors

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to the pathophysiology of asthma. Mast cell–derived PGE2 is also a potent inducer of smooth muscle contraction and vasodilation. Moreover, both PGE2 and the leukotrienes have been shown to attract leukocytes into the reaction site and to modulate a variety of cellular immune responses, particularly the increased production, or inhibition, of various cytokines.

C. Mediator Classes: Cytokines

A vast amount of information collected over the past 20 years has shown that certain pro-inflammatory cytokines play a significant role in the pathogenesis of chronic allergic inflammation and disease. None have gained more recognition than those commonly classified as the Th2 cytokines, which were originally reported to be produced by a subpopulation of mouse CD4+ lymphocytes (28) and presently include IL-4, IL-13, IL-5, IL-6, and IL-10. In contrast, the increased expression of so-called Th1 cytokines, which are exemplified by IFN-γ, IL-2, and IL-12, is negatively associated with allergic conditions. Of significant relevance, there is now striking evidence that FcεRI+ cells, particularly basophils, are important producers of specific Th2 cytokines—a finding that has reinforced the concept that these cells play a more pivotal role in the development of chronic disease than originally thought (29). Human basophils generate de novo and secrete large quantities of IL-4 and IL-13 following IgE crosslinking, with solid evidence that they have the capacity to produce more IL-4 than any other leukocyte circulating in blood (30–34). Studies show, in fact, that they can account for up to 10-fold more IL-4 than that made by T lymphocytes (35,36). This observation most likely reflects the notion that the frequency of antigen-specific basophils (i.e., those that express allergen-specific IgE) is far greater than the number of antigen-specific lymphocytes circulating in blood. Likewise, several studies report the production of Th2-type cytokines by human mast cells (37–41); however, controversy exists regarding the ability of these cells to generate Th2 cytokines, particularly IL-4. The evidence for mast cell–derived cytokines derives predominantly if not exclusively from immunohistochemistry techniques that detect cytokine-expressing cells in suspension or tissue that are co-staining either for intracellular tryptase or cell surface c-kit (CD117). In vitro studies using mast cells purified from skin or lung tissue have not been as supportive for mast cells being a source of cytokines (42). In fact, mast cells derived from cord blood precursors cultured in medium containing SCF and IL-6 have also challenged the immunohistochemistry data. These in vitro–derived cells, which stain for tryptase and are morphologically identical to tissue-derived mast cells, do not generate IL-4 mRNA or protein but do appear to make IL-5, a Th2-type cytokine that is not made by basophils (43). The controversies regarding the generation of cytokines by basophils and mast cells are unexpected given the fact that both cell types express FcεRI. While several explanations can account for such differ-

ences, there is evidence from studies using murine mast cells that indicate the ability to generate specific cytokines is determined by the level of FcεRI expression—a subject that will be discussed in greater detail below.

D. Relative Roles for Basophils/Mast Cells Versus Lymphocytes in Cytokine Secretion

By producing IL-4 and IL-13, basophils (and perhaps mast cells) may help orchestrate a variety of biological responses that are central to the pathogenesis of allergic disease. This belief is founded on the fact that these two cytokines induce isotype switching from IgM to IgE in B lymphocytes (44,45) and activate endothelium for eosinophil adhesion and transmigration by upregulating VCAM-1 expression (46–48). IL-4 is also critical for the development of the Th2 response (49,50). Interestingly, basophils and mast cells are among the few cell types that express the ligand for CD40 (CD40L) (51), which, in the presence of IL-4 or IL-13, provides the co-stimulus required for IgE synthesis by B cells. In fact, in vitro studies have shown that activated basophils alone can induce IgE synthesis by B lymphocytes (51,52). In the same studies, mast cells did not support the synthesis of IgE despite expressing CD40L. Once again, this latter finding challenges the concept that mast cells readily produce IL-4 and IL-13.

E. Sensitivity of Basophils and Mast Cells

An important component of the allergic response is the sensitivity of mast cells and basophils to stimulation through FcεRI. This aspect of the overall response is not often considered because determining sensitivity of the cells themselves in a given patient is difficult. Indeed, it is difficult to determine for the circulating basophil and currently not even feasible for the tissue mast cell. By sensitivity, we refer to the number of receptors that need to be aggregated in order to result in secretion that is 50% of a maximal response (53,54). Clearly, the ability of an antigen to initiate a response from the mast cell or basophil depends on how many receptors need to be activated. Therefore, the sensitivity of a cell to a particular concentration of antigen will be a function of at least eight identifiable parameters:

1. Number of epitopes on the antigen
2. Avidity of the antigen for its respective IgE
3. Concentration of antigen-specific IgE
4. Ratio of antigen-specific IgE to total IgE
5. Accessibility of IgE to the mast cell or basophil
6. Number of receptors per mast cell or basophil
7. Number of cell surface antigen-specific IgE molecules required to initiate secretion
8. Maximum secretory response

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The number of epitopes on the antigen and the avidity of the antigen for its respective IgE antibody are related since the avidity is a function of both single site affinity (i.e., the intrinsic affinity of a given antigen epitope for its specific antibody) and the number of epitopes that are involved in binding to either the same specificity antibody or a mix of antibodies that all bind to the same antigen. Parameters 1 and 2 determine the concentration of antigen required to reach an optimal number of crosslinks on the cell surface, i.e., weak avidities require more antigen. There are well-explored theoretical studies that show that the peak of the crosslinking curve for a small bivalent hapten occurs at 1/2Ka (55,56), where Ka is the single site affinity of the hapten for the antibody. In a similar, although more complicated manner, the optimal number of crosslinks is related to the avidity (57–59).

The next four factors partially determine how many molecules of antigen-specific IgE reside on the surface of the mast cell or basophil. For example, if a mast cell has 100,000 FcεRI receptors, the total IgE concentration is 1000 ng/mL, and the antigen specific IgE titer is 200 ng/mL, then 20,000 receptors will be occupied by the antigen-specific IgE (because the occupancy of the receptor is determined by the very high association constant between this receptor and IgE and when the IgE concentration is this high, it is simpler to state the numbers as if all receptors were occupied—the numbers will be valid within 2–3%). As will be explored in greater detail in the next section, the total IgE concentration also determines the expression of FcεRI, so that a ratio of specific to total IgE of 20%, as in the example above, has a far different consequence if the total IgE concentration is 10 ng/mL vs. 1000 ng/mL. Since the IgE concentration determines receptor expression, the ability of IgE antibody to be present outside the vascular compartment also determines the ability of mast cells to respond to antigen.

The last two parameters determine the ultimate response of the cell when all other factors are equal. It is now clear from studies of human basophils that the sensitivity of the cells varies markedly among individuals. The distribution of sensitivities among the population centers at a value of ~2000 antigen-specific IgE molecules per cell (54). In other words, 2000 antigen-specific IgE molecules need to be present for the cell to generate a response that is half of its maximum. The maximum itself appears to be an independent parameter of basophil function (54). For example, subjects can be found whose basophils are significantly more sensitive than the median, while their maximal response is 10% of what the cell is capable of secreting. With respect to the sensitivity distribution, low values (i.e., very sensitive cells) have been measured in the 200 IgE molecules per basophil range and highs have been measured above 30,000. These extremes are not common, with 90% of the population falling between 1,000 and 10,000.

The maximum secretory response is partially a function of the mediator being examined. Histamine release is often expressed as a percentage of the total

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cellular content since this is a preformed mediator. For this mediator, the maximum can only be 100% as there have been no demonstrations of histamine synthesis closely following stimulation. Defining the maximum for the other two major classes of mediators, newly synthesized arachidonic acid products or cytokines, is more difficult. However, there are non–IgE-dependent stimuli, both physiological and nonphysiological, that induce much greater levels of either LTC4 or IL-4 than can be obtained from IgE-mediated secretion. In this way, even the secretion of these two mediators can be placed in the context of a maximal response. Alternatively, histamine release can be expressed as ng per million cells in the same way that LTC4 and IL-4 are often expressed. In either case, these maximums vary considerably among basophil preparations. While there is a loose correlation among the secretion of histamine, LTC4 and IL-4, there are clearly factors independent of the initial signal strength that determine the amount of secretion of the three mediators. The mechanisms underlying the differences among subjects are not currently understood.

The current descriptions of sensitivity and maximal response are best applied to secretion of histamine. However, a more complete understanding will require further information about the same parameters in the context of lipid and cytokine release. Both of these mediators are critical determinants of the allergic inflammatory response, so that understanding the extent to which new therapeutics need to suppress basophil and mast cell responsiveness will be dependent on new information regarding the relative roles of each of the secreted mediators. Recent studies have indicated that basophil sensitivity is very similar when viewed from the secretion of each of three classes of mediators. This means that if cell surface antigen-specific IgE is present at a density sufficient to initiate only 50% of the maximum histamine release, approximately 50% of the maximal secretion of IL-4 and LTC4 will also be obtained. It remains to be determined whether the equivalent suppression of secretion for each of the three classes of mediators will lead to equivalent reductions for the functional endpoints of the three different mediator classes.

The above discussion is not simply an academic exercise in understanding the details of secretion. It has a very practical consequence for understanding the ability of some therapeutics to modulate the allergic reaction. This is particularly true for any therapy that operates by reducing the circulating IgE concentration. For example, consider an atopic donor with basophils (and perhaps mast cells) expressing 250,000 FcεRI receptors, a circulating IgE titer of 500 ng/mL, 20% of which is directed against ragweed antigen E (AgE), and a sensitivity of 1000 molecules of AgE-specific IgE (for one-half of their maximal response). Taking into account the binding affinity of IgE for its receptor, reducing the response of the basophils to less than 10% of their normal response, in the absence of a change in their FcεRI expression, would require a reduction in IgE titer between 2,000- and 10,000-fold (60). We do not yet know how far the basophil response must be suppressed to make a noticeable change in symptoms, but physiological

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studies in airway tissues indicate that local secretion of 2–3% of available tissue histamine leads to a maximal contraction of the smooth muscle tissue (61). It may be that not all tissue mast cells are secreting in these tissues hung in physiological baths for challenge, but this number does suggest that only very low levels of secretion are necessary for significant end-organ responsiveness. This amount of reduction seems extreme and partly results from the high affinity of IgE for FcεRI as well as the high sensitivity of the basophils and mast cells.

We now know, however, that the concentration of IgE antibody also determines the expression of FcεRI on basophils and mast cells (see below), although a direct demonstration of this observation on human tissue mast cells has not yet been made. Thus, if IgE titers fall, so too does FcεRI expression. This dynamic considerably alters the prediction made above. Much more modest and achievable changes in IgE titer, 50- to 100-fold, significantly reduce the function of basophils. The details of this prediction are borne out in therapeutic trials of anti-IgE antibody.

Much of our information for human cells comes from studies of basophils, as these cells are more readily accessible. Limited studies of tissue mast cells have been done, and thus far some of the general features of the basophil translate well to the mast cell. In particular, mast cell sensitivity appears to be similar to basophils, i.e., only a few thousand antigen-specific IgE molecules per mast cell, or less, are required for significant activation. However, one curious distinction is that most mast cell preparations, including those derived from BAL washings, express low numbers of receptors. For basophils, the median level of expression is 150,000 FcεRI per basophil, ranging from 5000 to nearly one million among preparations. Most atopics express greater than 200,000 (presumably due to their higher circulating IgE titer). In our studies of mast cells, most densities fall below 30,000 per mast cell, with only 25% of the mast cell preparations showing values well above 100,000 (62). Indeed, because flow cytometry is not very sensitive when working with impure cell populations and because the contaminating cells in mast cell preparations cause a higher than usual control fluorescence, mast cells often do not even have measurable levels of FcεRI using these methods. Based on various calibrations, the flow cytometer probably cannot detect below 10,000 receptors in these preparations. The significance of these observations to therapeutics that reduce IgE antibody is not yet clear, but at face value might indicate that reduction of mast cell IgE to levels insufficient to allow antigen-induced secretion may be easier than reduction of cell surface basophil IgE.

III. FcεRI Expression on Basophils and Mast Cells

A. Descriptives

A hallmark of the basophil and mast cell phenotype is the expression of FcεRI, the high-affinity receptor for IgE antibody. In the late 1970s it was observed that

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there was a strong correlation between the serum IgE concentration and the cell surface expression of FcεRI on circulating basophils (63). It was not possible at that time to determine if the two parameters were linked by some preexisting co-expression mechanism (e.g., genetic linkage) or whether the expression of one determined the expression of the other. With improvements in technology it became possible to discriminate between these two possibilities. It is now accepted that the presence or absence of IgE antibody determines the relative expression of the receptor. This has been demonstrated by a variety of methods in both mice and humans (60,64–68). In mice, it is possible to demonstrate that increasing the concentration of IgE, either in culture or in vivo, upregulates the expression of FcεRI on both mast cells and basophils. Similar results have been obtained for human basophils, both in vitro and in vivo. Both increases and decreases in IgE have been explored in the human basophil studies, and both conditions alter FcεRI expression, either up or down, as appropriate to the concentration of IgE. A caveat for the studies of human basophils in vivo is that the decrease in IgE antibody was induced by the administration of an anti-IgE antibody, so it remains possible that this reagent had unrecognized direct effects on FcεRI expression. This connection between IgE titer and FcεRI expression establishes an interesting feedback loop under conditions in which IgE titers are being altered. It appears as a positive feedback loop which markedly enhances the cellular responsiveness when IgE concentrations rise. From one perspective, such upregulation of FcεRI allows a cell to maintain a level of functionality against one antigen even if new antigenic specificities of IgE appear and cause an increase in the total IgE titer. In the absence of this feedback loop, the addition of new antigenic specificities might actually lead to a decrease in the response to preexisting antigens. One interesting speculation relates to the teleological explanations for the presence of this feedback loop. If parasitic infections induce a nonspecific rise in IgE in the absence of the IgE-receptor feedback loop, mast cell/basophil responses to the parasite antigens would diminish. To compensate, the immune system developed the feedback loop to offset this effect. Whatever the reasons for the existence of this feedback loop, it provides a useful biological side effect for therapeutics that act to decrease circulating IgE antibody levels.

B. Mechanisms

In vitro studies of human basophils indicate that regulation does not occur through another cell type, i.e., it appears that IgE directly influences basophils to express more FcεRI (65). Until recently, it was not clear whether IgE influenced FcεRI expression by direct interaction with FcεRI itself because the concentration dependence of FcεRI upregulation by IgE suggested that IgE may interact with another IgE binding molecule. The EC50 for upregulation occurs at approximately 250 ng/mL (~1 nM), which is significantly higher than estimates of the

affinity of IgE for FcεRI, which should range from 3 to 25 ng/mL. On the other hand, the EC50 was too low to readily fit the interaction of IgE with other known IgE-binding molecules like CD32, CD23, or galectin-3. However, recent direct studies demonstrated that neither IgG nor lactose influenced upregulation by IgE, indicating no role for either CD32 or galectin-3. Basophils do not express CD23 protein or mRNA, and IgE upregulation was not influenced by MHM6, an antibody known to bind to CD23 and alter IgE binding. Finally, a mutated form of the Fc portion of IgE (R334S), which has been demonstrated to bind to FcεRIα with 10- to 100-fold lower affinity than the unaltered Fc molecule, was found to have a 30-fold lower potency than the parent molecule for upregulating FcεRI expression. Parenthetically, the mutant IgE-Fc had binding characteristics similar to the parent when tested against binding to CD23 (FcεRII). These studies cumulatively demonstrated that IgE probably interacts with FcεRIα itself to upregulate FcεRI expression (69).

A central question is whether the presence of IgE induces either mRNA and/or protein synthesis and, conversely, whether the absence of IgE leads to downregulation of either of these events. A direct answer is not currently known. However, there are various clues that suggest that synthesis may be constitutive. Furthermore, these changes do not appear to be due to receptor recycling. A variety of studies in RBL cells indicated that a small amount of receptor expression could be controlled by receptor cycling (70,71). In the absence of IgE, cell surface receptors would be internalized, only to be reaccumulated on the cell surface if IgE was present. In other words, IgE, when bound to the receptor, appeared to stop an otherwise continuous loop of recycling. Under these conditions, two- to threefold increases in cell surface receptor expression could be observed when IgE was added to the medium, without the synthesis of any new receptors. It is often the case that a one-week incubation of human basophils leads only to two- to threefold increases in cell surface receptors, so the RBL results provided a viable explanation for the human basophil phenomenon. However, in vitro studies indicated that the amount of FcεRIα obtained from a whole cell lysate increased or decreased in concert with cell surface expression. This indicated that there was not a store of receptor accounting for the changes. An additional caveat is that although in vitro experiments tend to result in modest changes, there are examples of 20-fold increases that are not as readily explained by a cycling mechanism similar to that studied in RBL cells. When considering that the expression in vivo ranges 200-fold, it seems even less likely that a cycling mechanism could be operative. Thus, receptor is synthesized during upregulation and lost during downregulation. It remains to be determined whether these are induced events. If IgE interacts through FcεRIα and induction occurs, this would be a new property of this well-studied receptor, i.e., the ability for univalent binding to induce a signal within the cell (the signal to make more receptor). These studies have been difficult to do in human basophils because receptor

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upregulation appears very slow. A loose calculation indicates that 400–600 receptors are placed on the cell surface per hour under optimal in vitro conditions. This makes pulse-chase studies demanding. However, changes in the expression of mRNA for FcεRIα are feasible, and these studies, carried out a with a variety of detection methodologies, indicate that no changes occur withing the first day or two following exposure to high concentrations of IgE antibody (unpublished observations). While it remains possible that protein synthesis is induced in the absence of changes in mRNA or that mRNA changes occur much later than thus far examined, an alternative hypothesis is that synthesis is constitutive and that maintenance on the cell surface is dependent on occupancy by IgE. This scheme is similar to that of CD23, where a cell surface metalloprotease clips the receptor if it remains unoccupied by IgE. In the case of FcεRIα, the mechanism of removal could be similar to CD23 or related to some endocytotic process. The consequence is that receptor is made at a slow rate, and if occupied rapidly enough by IgE, it is protected from removal and therefore accumulates on the cell surface over time. With this scheme, receptor occupancy is determined by the forward rate constant of IgE binding rather than by equilibrium (because the off rate is so slow for this receptor). The forward rate constant of IgE binding to whole cells is known to be quite slow so it is possible to explain the modest shift in the EC50 for upregulation by the slow forward rate of occupancy of FcεRIα by IgE (65,69). In this scheme there is no endpoint, except the limits of protein crowding on the cell surface, but this is not much of a concern, at least for basophils, because they do not have a long life span. The longest life span estimates have been 2 weeks, which is not enough time, given the rates of synthesis projected from in vitro studies, to express more than several hundred thousand receptors. However, mast cells are believed to survive for months, so that an open-ended upregulation model may be too simplistic.

C. Regulation and Genetics

As will be noted in the studies below, expression of FcεRI may be a critical determinant in the success of therapies that suppress free circulating IgE levels. Therefore, understanding how the expression of the receptor is regulated may suggest additional ways to augment such therapies. Understanding the mechanism of its regulation by IgE antibody itself is important, but defining other intrinsic and extrinsic modulators of its expression will also be relevant. A variety of recent studies have focused on the expression of FcεRI during mast cell maturation. This kind of information will be important, but it would not be surprising if it does not strictly apply to regulation that occurs in a fully mature cell. For example, it appears that IL-4 may alter the expression of FcεRI in developing mast cells, but its effects appear to depend on the precise source or conditions of mast cell culture (72–74). This receptor is expressed early in the differentiation

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of mast cells at a time reasonably coincident with the expression of elements necessary for granule formation (75). As this differentiation process is influenced by a variety of cytokines, it is not surprising that expression of the receptor is also affected. It remains to be determined if there are ligands (that are not IgE) that specifically alter FcεRI expression independent of effects on other aspects of the development process. With respect to IL-4, we have found that expression of FcεRIα on mature circulating basophils is not influenced by this cytokine in either the presence or the absence of IgE antibody (unpublished observations).

Following the initial description of the sequence of the gene for FcεRIα and its 5′ upstream region (76), there has been little information regarding the responsive transcription elements in this gene. However, one recent study (77) has demonstrated the presence of two *trans*-acting protein response elements in the FcεRIα promoter as defined in reporter constructs. Ets and GATA *cis*-acting response elements exist in the human promoter region (using 5′ sequences approximately −75 bp 5′ of the initiation site), and antibodies to GATA-1 and Elf-1 induce a supershift of mixtures of mast cell nuclear extracts and probe in EMSA tests. Interestingly, GATA-1 has been demonstrated to be required for expression of the mast cell granule enzyme carboxypeptidase A (78), further linking the expression of these two mast cell genes. No information is yet available about response elements that relate to IL-4 stimulation or other known cytokine-stimulated transcription factors.

D. Functional Consequences of Receptor Regulation

In human cells, only the increase and decrease in mass of FcεRIα during up- or downregulation of FcεRIα due to the presence or absence of IgE has been demonstrated. In murine studies, expression of FcεRIβ has also been observed (67), but this subunit is required for expression of FcεRI in murine cells (79). In human cells, only FcεRIα and γ are necessary for expression (80–82), so it remains possible that there is discordant regulation of the various FcεRI subunits in human cells. The fact that expression of FcεRIβ is a strong determinant of the signal developed in an aggregated receptor suggests (83) that coordinated expression of all receptor subunits would be necessary to produce cells that were actually more sensitive to stimulation. This coordinated upregulation would apply to other early signaling components that may be found to be rate-limiting. Recent in vitro functional studies of FcεRI upregulation in human basophils in fact suggest that coordinated upregulation of the necessary elements of the IgE-mediated signaling pathway does not always occur. The elements that may limit upregulation of function have not been identified. The instances of uncoordinated upregulation of signaling component expression occur in studies of human basophils under culture conditions and may therefore not occur as readily in vivo. Nevertheless, the fact that uncoordinated upregulation can occur may indicate that there

are multiple conditions that determine the functional consequence of FcεRIα upregulation induced by IgE. Much more study is required to sort out these observations and their implications to pathophysiology and therapeutics.

E. Relationship of Basophil Characteristics to Other Leukocytes

As mentioned above, among circulating leukocytes a correlation between surface expression of FcεRI on basophils and levels of serum IgE levels has been known for nearly two decades (63). This original observation involved basophils of atopic individuals and has been recently confirmed (84). Recently, we examined this relationship in basophils, monocytes, and eosinophils of individuals with a variety of disease states characterized by an elevation of IgE, including allergic asthma, atopic dermatitis, hypereosinophilic syndrome, hyper IgE syndrome, helminthic-infested hosts, and IgE myeloma (85). Within a range in serum IgE levels ($3–4 \times 10^6$ ng/mL) and diverse diseases states, the relationship of serum IgE to basophil FcεRIα expression persists. Taken together with the effects of anti-IgE infusion on human basophil FcεRI expression (see below), there is increasing evidence for serum IgE regulation of basophil expression of its high-affinity receptor.

Accumulating evidence from several groups suggests the presence of FcεRI on other leukocytes including circulating blood monocytes, eosinophils, and platelets. Whereas FcεRI is readily detected on the surface of blood basophils of nearly all individuals, including those with low serum IgE, the observed expression of the high-affinity IgE receptor on other leukocytes has been more difficult in terms of disease state and levels of expression. In the case of blood monocytes, FcεRIα expression has been reported in allergic diseases such as rhinitis, asthma, and eczema with a positive correlation to serum IgE (84,86). The highest level of FcεRIα expression was observed on monocytes of subjects with atopic dermatitis (86); however, the overall level of monocyte expression is typically 10- to 100-fold lower than that found on basophils from the same individual. In our preliminary studies, monocyte FcεRIα has not been detectable in nonatopic, elevated IgE states, suggesting that the presence of IgE is not a sufficient regulatory stimulus for FcεRI expression on monocytes as it is on basophils (65). One lab has detected FcεRIα expression on monocytes of nonatopic individuals but no IgE. They also confirmed that there are higher levels of FcεRIα and IgE expression on monocytes of atopic subjects (87). An explanation for monocyte expression of FcεRI in atopics, and the reduced level relative to the basophil, has not been found.

An important difference from basophils is that monocyte FcεRI complexes lack the β subunit and are composed only of an α chain and two γ chains. The existence of such an alternate FcεRI receptor complex was first demonstrated by in vitro transfection experiments that demonstrated stability of the human αγ2

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complex, a feature not shared by murine or rodent FcεRI complexes (80). In terms of function, FcεRI-bearing monocytes have been shown in vitro to present IgE-targeted antigens to T cells at significantly lower concentrations (10- to 1000-fold) than required for traditional IgG-facilitated antigen capture (88). FcεRI can also activate monocyte PGE2 release (89) and a calcium signal (86). However, the exact function of FcεRI on monocytes remains uncertain.

Surface FcεRI expression on peripheral blood eosinophils has been variably observed in vivo since the first report of the high-affinity receptor on blood eosinophils (90) and remains a topic of controversy (91). The initial description involved blood eosinophils from subjects with hypereosinophilic syndromes (90), but subsequent studies have failed to reproduce these observations (85,92). Blood eosinophils express both mRNA and intracellular protein for the α and γ subunits of the FcεRI receptor complex but lack the β subunit protein. Eosinophils in culture with IL-5 release the α subunit protein into the culture supernatant, but the addition of IgE failed to induce expression of the receptor on the cell surface (92). In addition, culture of eosinophils with IL-4 is reported to augment mRNA and protein for α chain without significant alteration of surface α expression (93). The presence of intracellular pools of α protein and the secretion of the α subunit by cultured eosinophils may offer one explanation for the immunodetection of eosinophil FcεRI α in tissue biopsies of late-phase allergen challenge of the nose (94) and skin (95). Indeed, in the study by Sihra et al. (84), the levels of eosinophil expression of FcεRIα were even lower than observed on corresponding monocytes, if detected at all, and were not correlated to serum IgE levels. Another study of peripheral blood eosinophil FcεRIα expression in subjects with allergic rhinitis suggested levels of approximately 0.5% of basophils and failed to show significant induction of eosinophil superoxide release, degranulation, or LTC4 production following IgE-dependent activation (96). Thus, in our hands and others, significant surface expression of the high-affinity receptor on peripheral blood eosinophils has not been confirmed.

Other leukocytes reported to bear surface FcεRI include tissue-derived Langerhans cells (97,98), where surface α chain expression is also quite variable. Surface expression of FcεRI on Langerhans cells appears to be limited by the amount of gamma chain expressed and is greatest in individuals with atopic dermatitis (99). Peripheral blood dendritic cells (100) have also been shown to bear the alternate form of the high-affinity IgE receptor and may use it for allergen presentation to T cells. Platelets have also been reported to bear FcεRI and release inflammatory products such as RANTES after triggering of the receptor (101,102). In summary, beyond the well-characterized function of the receptor on circulating basophils, the role of the high-affinity receptor on other bloodborne cells and factors regulating its expression on these cells are not yet firmly established.

IV. In Vivo Regulation of FcεRI on Human Basophils

A. Studies of Anti-IgE Antibody

The design of a humanized antibody (rhumAb-E25) that could bind IgE and prevent its attachment to the high-affinity receptor, without the harmful side effect of inducing receptor crosslinking, has allowed a novel means of interfering with allergic responses (103). Simultaneous E25 infusion in mice undergoing allergen sensitization demonstrated significant reductions in both total and allergen-specific IgE, possibly through depletion of CD23-positive IgE-bearing B cells (104). In humans, therapy with anti-IgE in allergic asthmatics showed reductions in the early phase (105) and late phase (106) responses to airway allergen challenge and reductions in sputum eosinophils.

Studies of basophil FcεRIα in the context of anti-IgE therapy allowed a unique opportunity to explore the effects of IgE modulation on receptor expression. As part of a phase I trial with this monoclonal antibody, allergic subjects were infused with doses of E25 antibody based on both body weight and serum IgE biweekly for one year. Examination of free IgE titers 2 hours after the initial antibody infusion revealed a 99% decline in free IgE that was maintained by subsequent bi-weekly infusion. Both basophil surface-bound IgE and FcεRIα staining by flow cytometry revealed a 50% reduction 3 days after the initial infusion and 90–100% reduction approximately 1 week after the initial E25 infusion (60). The reduction in IgE density on basophils at 3 months of biweekly infusions was greater than 99% (average 2,200 molecules per basophil at 3 months vs. average 240,000 molecules per basophil at baseline). Total IgE receptors were likewise reduced by 97% at 3 months (8600 molecules per basophil) resulting in threefold more unoccupied receptors than occupied ones (Fig. 2).

The functional consequences of basophil receptor modulation after 3 months of anti-IgE infusion in these subjects was determined by in vitro basophil histamine release responses to three secretagogues: antigen (*Dermatophagoides farinae*), to which all subjects had skin test sensitivity; polyclonal anti-IgE; and fMLP, a non–IgE-dependent stimulus for basophil histamine release. A 90% reduction in histamine release triggered by an optimal dose of *D. farinae* was seen after 3 months of anti-IgE (Fig. 2). In contrast, polyclonal anti-IgE–mediated histamine release was more modestly reduced at 40% of baseline values without a shift in the dose-response curve. As expected, no change was seen in terms of fMLP-mediated histamine release. In a subset of subjects, sensitization of the open receptors on basophils with benzyl-penicilloyl (BPO)-IgE, at this point in the treatment, followed by either antigen-triggering (BPO-HSA) or by polyclonal IgE was sufficient to restore responses to pretreatment levels seen with *D. farinae* or polyclonal anti-IgE. The observation suggested that despite a 97% decline in IgE and FcεRI at 3 months, a sufficient number remained to be sensitized and capable of initiating normal mediator release to an IgE-triggered event. In addi-

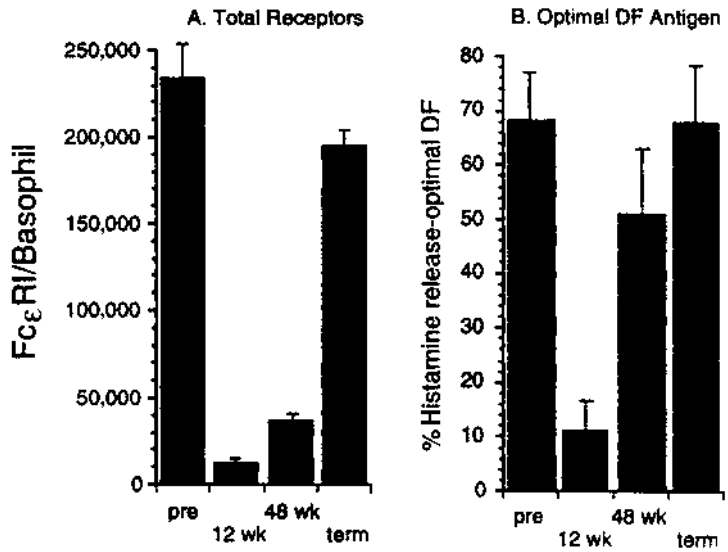

Figure 2 Quantitation of basophil total IgE receptors by lactic acid elution and *D. farinae* (DF) and anti-IgE–induced basophil histamine release responses. (A) Mean RIST values for total IgE eluted from subjects basophils ($n = 11$) prior to infusion of E25 (pre), after 12 weeks of rhuMAb-E25 treatment (12 weeks of E25), one hour after last infusion of E25 (46 weeks of E25), and at termination from the postinfusion follow-up study (termination of post E25 study). Values are expressed as the mean ± SEM number of receptors per basophil. (B) Samples obtained at the same timepoints as in Figure 3 were examined for histamine release responses to optimal doses of *D. farinae* or anti-IgE. Results are expressed as mean percent of histamine release. (From Ref. 68.)

tion, skin prick test titrations to *D. farinae* after 26 weeks of E25 infusions were significantly suppressed as compared to baseline and thus support E25 modulation of skin mast cell functional responses (107).

The implication of these functional studies is that shifts in receptor occupancy by allergen-specific IgE could restore basophil mediator release. Further support for this hypothesis was obtained from later portions of the same phase I E25 trial. After 28 weeks of biweekly infusions, subjects were randomized to receive a 3 to 20-fold reduction in E25 dosing and continued for another 18 weeks. The result of this reduction was an increase reflected in free serum IgE levels from a mean of 7 ng/mL at week 28 to approximately 21 ng/mL at the termination of infusions (68). By the end of this treatment period, the levels of basophil surface IgE expression and FcεRIα rose three- to fourfold, with the average number of receptors per basophil rising to nearly 35,000 per cell at the

time of termination of E25 dosing (Fig. 2). In terms of functional impact, allergen-dependent (*D. farinae*) basophil histamine release was nearly restored and polyclonal anti-IgE–induced release was completely restored by the time of the final E25 infusion (85). Thus, a slight reduction in E25 dosing allowed an increase of 10–20 ng/mL in free serum IgE, and this resulted in repopulation of the basophil surface with sufficient allergen-specific IgE and receptor numbers to reverse the previous reduction in histamine release responses. The clinical impact of the recovery of histamine release responses was not examined.

In the last part of this study, subjects were followed for up to one year after their final dose of E25 to examine restoration of serum IgE levels, basophil phenotype, and basophil function (68). As previously noted, due to the dosage reduction introduced in the latter half of infusions, basophil function was nearly restored, with only a slight rise in circulating free IgE and basophil receptor numbers by the last day of infusion. During the first 8 weeks post-treatment, free IgE levels rose nearly fivefold, while basophil surface IgE and FcεRIα rose 2.7-fold and 2-fold, respectively (Fig. 3).

B. In Vitro Regulation of FcεRI on Human Basophils from Treated Subjects

The implication that free serum IgE was modulating basophil surface phenotype in vivo in E25 recipients was tested in parallel in vitro experiments using basophils from the patients in the phase I study. Basophils were isolated from subjects within 8 weeks of their last E25 infusion, when they had low receptor number and surface-bound IgE. Enriched basophils were then cultured in the presence of IgE for up to 7 days and observed to have a near twofold rise in both surface-bound IgE and FcεRIα expression (68). The degree of FcεRIα enhancement varied inversely with the baseline expression levels on basophils with a range of 0.7- to 15-fold. The kinetics of receptor enhancement in vitro appeared accelerated versus the in vivo rate. The variation in rates may be due to higher levels of IgE used with basophils in vitro (500 ng/mL) versus the mean IgE level seen at 8 weeks postdiscontinuation (94 ng/ml; see Fig. 3). In support of this concept is the fact that dose dependence for IgE-dependent FcεRIα enhancement was seen in parallel cultures using 50 and 500 ng/mL IgE. Another effect of IgE exposure in vitro appeared to be maintenance of surface-bound IgE on basophils since cultures without IgE demonstrated a decline in levels after 7 days. In later studies, prolonged culture of basophils in the absence of IgE led to a loss of both surface-bound IgE and receptor levels (65).

The in vitro alterations in basophil IgE receptor phenotype were not observed in basophils cultured in medium alone or in the presence of similar amounts of IgG. A potential role for CD23 and FcγRII in regards to binding of IgE complexes was examined in basophil cultures. No alteration in basophil

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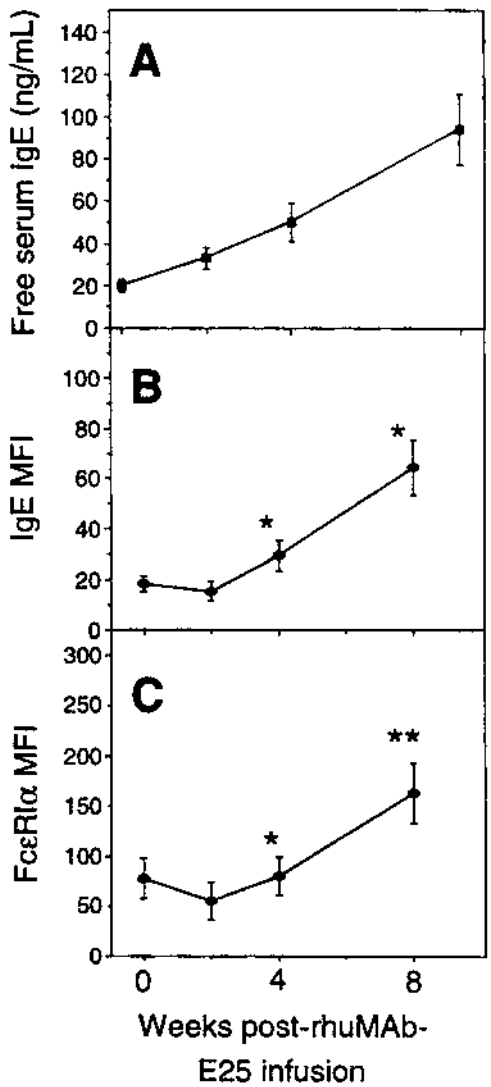

Figure 3 Levels of free serum IgE (A), basophil surface IgE (B), and FcεRIα (C) within 8 weeks after discontinuation of rhuMAb-E25. (A) Mean free IgE values ± SEM for 11 subjects are shown. Flow cytometric analysis of blood basophils by FITC-conjugated goat antihuman IgE (B) and by FcεRIα Mab 22E7 plus PE-conjugated goat antimouse IgG (C). Values represent mean ± SEM for 11 subjects (preinfusion mean levels are 216 ± 5 MFI for IgE). $*p < 0.01$ or $**p < 0.001$ as compared to day 0. (From Ref. 68.)

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expression of FcγRII was seen under any culture condition, and CD23 was never detected. As noted previously, basophil culture experiments have shown a need for IgE binding to its high-affinity receptor to achieve enhancement, although the mechanisms remain unknown (65). Together, the in vivo and in vitro data strongly support a role for IgE regulation of basophil FcεRI expression; however, the mechanism remains uncertain. Further, the expression and regulatory factors for FcεRI expression on other leukocytes remain to be established.

C. Implications of In Vivo Results for Therapeutic Dosing of Anti-IgE Antibody

The in vivo downregulation of FcεRIα during treatment with anti-IgE antibody yielded two important observations. First, in the study described, the reduction of free IgE was just sufficient to reduce basophil responsiveness to its threshold. Due to the limit of detection, we could not directly determine the endogenous cell surface expression of IgE on many of the treated subjects' basophils. Estimates indicate that densities were often below 2500 molecules per cell. Based on previous studies of basophil sensitivity in the general population and the fact that polyclonal anti-IgE antibody continued to induce, on average, maximum histamine release that was 50% of the pretreatment response, these results indicated that cell surface IgE density hovered around 1000–2000 molecules per cell. Therefore, the antigen-specific IgE was probably below 500 molecules per cell, accounting for the very weak responses to *D. farinae* antigen. The observation that sensitizing the cells with antigen-specific IgE restored responsiveness also suggested that suppression of the response was borderline. This impression was supported by the second observation: a relatively small increase in cell surface receptors, which followed a modest reduction in the amount of E25 antibody used for treatment, leading to a slight increase in free IgE, caused a marked restoration of the functional response. Thus, for this population of patients, with starting IgE titers around 500 ng/mL, 100-fold reductions are on the threshold of sufficiency. This conclusion led to suggestions concerning the nature of patients that can be effectively treated.

Using the functional study as a guideline, a somewhat counterintuitive suggestion arises. Two key parameters in the effectiveness of a treatment that produces conditions that are marginally sufficient to reduce basophil function are the ratio of specific to total IgE and the total IgE level. However, if we consider an average IgE titer of 500–1000 ng/mL for atopic patients, the remaining element, ratio of specific to total IgE, becomes very important. As noted previously, some antigens are capable of driving an immune response which is heavily dominated by antigen-specific IgE. Thus, a patient with a single hypersensitivity may not be the best type of patient to treat unless they begin with a very low total IgE titer. For more typical levels of IgE antibody, the best patient may be one

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with multiple hypersensitivities. The logic is that any one allergen-specific IgE would represent a smaller fraction of the total IgE so that when cell surface IgE levels were suppressed to what would be considered the threshold for anti-IgE-induced histamine release, any given specific antigen would generate no response. Figure 4 shows an unpublished observation important in this context. For the study population used in the phase I trials of E25, the ratio of *D. farinae*–specific IgE to total IgE concentration is plotted along the abscissa, one symbol per patient. On the ordinate is the ratio of the *D. farinae*–specific response after and before E25 treatment (3 months into treatment). For this small study population, there is a reasonable correlation between the two parameters, suggesting that those with higher specific-to-total IgE ratios are less successfully treated under the protocol of the phase I study.

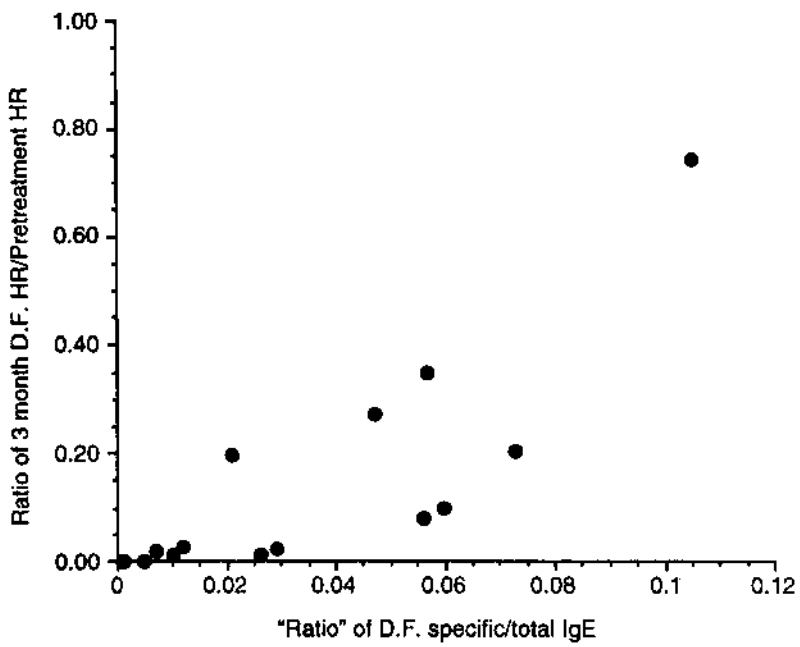

Figure 4 Relationship between the ratio of pretreatment *D. farinae*–specific IgE to total IgE and the change in basophil response following treatment with rhuMAb-E25. The ratio of specific to total IgE is not absolute, the assay for *D. farinae*-specific IgE has not been calibrated against standard IgE preparations. The output measure from this assay is in KIU per mL of serum. The basophil response is expressed as the ratio of posttreatment to pretreatment histamine release: basophils were from whole blood by Percoll single step gradients and challenged in vitro with an optimal concentration of *D. farinae* antigen.

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There are four important caveats to the above prediction. The first is trivial. If the doses of E25 used during treatment are very high, and IgE levels are suppressed far below those previously observed ($\gg$100-fold), then all these considerations are academic because these studies adequately demonstrated that elimination of IgE translates to elimination of allergic responses. The second caveat relates to the total IgE titer. For a given dosing regimen, clearly those with low IgE titers should be more successfully treated than those with very high titers. The third point is more subtle and may be an important consideration for those patients who do not seem to respond well to any treatment that only partially reduces free IgE titers. As noted previously, the sensitivity of basophils to IgE-mediated stimulation ranges 100-fold in the general population (although only 10-fold among the majority of subjects). Reducing total cell surface IgE to 1500 molecules per cell may have a significant impact on the average subject's basophil response but little impact when the cell is capable of responding well with only 200 molecules. From previous population studies, $\sim$10% of subjects have basophils with this high level of sensitivity. The typical treatment may simply be insufficient when the cells are this sensitive to stimulation. The opposite end of the spectrum would provide the best case for treatment. The fourth point concerns the relationship between the characteristics of basophils versus mast cells. As the tissue response probably depends on the response of the mast cell rather than the basophil, a great deal of this information about basophil behavior may not be entirely applicable to mast cells. There are indications that mast cells are similarly sensitive to stimulation, but the precise nature and dynamics of their receptor expression, and the influence of IgE, are not well studied. Thus, many of the arguments put forth may apply in a theoretical sense but at the practical level need refinement when being applied to mast cells. The various predictions about the requirements for successful anti-IgE antibody therapy might need significant modification because the setpoints and dynamics may differ for tissue mast cells.

V. Conclusion

In recent years, the role of mast cells and basophils in allergic disease pathogenesis has expanded. While their critical participation in anaphylactic events, via rapid release of preformed vasoactive mediators, remains firmly established, it is now realized that these cells can contribute prominently to subsequent inflammatory events by generating and releasing impressive amounts of lipid and cytokine mediators. This has served to underscore the need for novel therapeutics that target these cells and prevent them from being activated by allergen. At the forefront of these efforts are those that have the potential capacity to disarm mast cells and basophils by preventing their sensitization with IgE antibody. Indeed, anti-IgE antibody is now in late stages of clinical trials (60,68,106–110). Already

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this agent has proven to be an exceptional tool for exploring not only the role of IgE in allergic diseases but also its ability to regulate levels of FcεRI and IgE-mediated degranulation responses. It is also hoped that the future availability of these and other novel agents that target mast cells and basophils will ultimately lead to improved management of allergic disorders.

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References

1. Guo C-B, Liu MC, Galli SJ, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Identification of IgE-bearing cells in the late phase response to antigen in the lung as basophils. Am J Respir Cell Mol Biol 1993; 10:384–390.
2. Bascom R, Wachs M, Naclerio RM, Pipkorn U, Galli SJ, Lichtenstein LM. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. J Allergy Clin Immunol 1988; 81:580–589.
3. Charlesworth EN, Iliopoulos O, MacDonald SM, Kagey SA, Lichtenstein LM. Cells and secretagogues involved in the human late-phase response. Int Arch Allergy Appl Immunol 1989; 88:50–53.
4. Galli SJ. New concepts about the mast cell. N Engl J Med 1993; 328:257–265.
5. Dvorak AM. Cell biology of the basophil. Int Rev Cytol 1998; 180:87–236.
6. Dvorak AM, Saito H, Estrella P, Kissell S, Arai N, Ishizaka T. Ultrastructure of eosinophils and basophils stimulated to develop in cord blood mononuclear cell cultures containing recombinant human interleukin-5 or interleukin-3. Lab Invest 1989; 61:116–132.
7. Ottmann OG, Abboud M, Welte K, Souza LM, Pelus LM. Stimulation of human hematopoietic progenitor cell proliferation and differentiation by recombinant interleukin 3. Comparison and interactions with recombinant human granulocyte-macrophage and granulocyte colony-stimulating factors. Exp Hematol 1989; 17: 191–197.
8. Saito H, Ebisawa M, Tachimoto H, Shichijo M, Fukagawa K, Matsumoto K, Iikura Y, Awaji T, Tsujimoto G, Yanagida M, Uzumaki H, Takahashi G, Tsuji K, Nakahata T. Selective growth of human mast cell induced by steel factor, IL-6, and prostaglandin E2 from cord blood mononuclear cells. J Immunol 1996; 157:343–350.
9. Kirshenbaum A, Goff J, Kessler S, Mican J, Zsebo K, Metcalfe D. Effect of IL-3 and stem cell factor on the appearence of human basophils and mast cells from CD34+ pluripotent progenitor cells. J Immunol 1992; 148:772–777.
10. Mitsui H, Furitsu T, Dvorak AM, Irani AA, Schwartz LB, Inagaki N, Takei M,

Ishizaki K, Zsebo KM, Gillis S, Ishizaka T. Development of human mast cells from umbilical cord blood cells by recombinant and murine c-kit ligand. Proc Natl Acad Sci USA 1993; 90:735–739.

11. Valent P, Spanblochl E, Sperr WR, Sillaber C, Zsebo KM, Agis H, Strobl H, Geissler K, Bettelheim P, Lechner K. Induction of differentiation of human mast cells from bone marrow and peripheral blood mononuclear cells by recombinant human stem cell factor/*kit*-ligand in long-term culture. Blood 1992; 80:2237–2245.
12. Costa JJ, Demetri GD, Harrist TJ, Dvorak AM, Hayes DF, Merica EA, Menchaca DM, Gringeri AJ, Schwartz LB, Galli SJ. Recombinant human stem cell factor (kit ligand) promotes human mast cell and melanocyte hyperplasia and functional activation in vivo. J Exp Med 1996; 183:2681–2686.
13. Toru H, Eguchi M, Matsumoto R, Yanagida M, Yata J, Nakahata T. Interleukin-4 promotes the development of tryptase and chymase double-positive human mast cells accompanied by cell maturation. Blood 1998; 91:187–195.
14. Sawai N, Koike K, Mwamtemi HH, Kinoshita T, Kurokawa Y, Sakashita K, Higuchi T, Takeuchi K, Shiohara M, Kamijo T, Ito S, Kato T, Miyazaki H, Yamashita T, Komiyama A. Thrombopoietin augments stem cell factor-dependent growth of human mast cells from bone marrow multipotential hematopoietic progenitors. Blood 1999; 93:3703–3712.
15. Bochner BS. Basophils. In: Frank MM, Austen KF, Claman HN Unanue ER, eds. Samter's Immunological Diseases, 5th ed. Boston: Little, Brown, and Company, 1995:259–266.
16. Plaut M, Lichtenstein LM, Henney CS. Increase in histamine receptors on thymus-derived effector lymphocytes during the primary immune response to alloantigens. Nature 1973; 244:284–287.
17. Rocklin RE, Habarek-Davidson A. Pharmacologic modulation in vitro of human histamine-induced suppresor cell activity. Int J Immunopharmacol 1984; 6:179–186.
18. Griswold DE, Alessi S, Badger AM, Poste G, Hanna N. Differential sensitivity of T suppressor cell expression to inhibition by histamine type 2 receptor antagonists. J Immunol 1986; 137:1811–1815.
19. White WB, Ballow M. Modulation of suppressor-cell activity by cimetidine in patients with common variable hypogammaglobulinemia. N Engl J Med 1985; 312: 198–202.
20. Elenkov IJ, Webster E, Papanicolaou DA, Fleisher TA, Chrousos GP, Wilder RL. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. J Immunol 1998; 161:2586–2593.
21. Lagier B, Lebel B, Bousquet J, Pene J. Different modulation by histamine of IL-4 and interferon-γ (IFN-γ) release according to the phenotype of human Th0, Th1 and Th2 clones. Clin Exp Immunol 1997; 108:545–551.
22. Miller JS, Moxley G, Schwartz LB. Cloning and characterization of a second complementary DNA for human tryptase. J Clin Invest 1990; 86:864–870.
23. Vanderslice PS, Ballinger SM, Tam EK, Goldstein SM, Craik CS, Caughey GH. Human mast cell tryptase: multiple cDNAs and genes reveal a multigene serine protease family. Proc Natl Acad Sci USA 1990; 87:3811–3815.

MARCEL DEKKER, INC.
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24. Castells MC, Irani AM, Schwartz LB. Evaluation of human peripheral blood leukocytes for mast cell tryptase. J Immunol 1987; 138:2184–2189.
25. Xia HZ, Kepley CL, Sakai K, Chelliah J, Irani AA, Schwartz LB. Quantitation of tryptase, chymase, FcεRIα, and FcεRIγ messenger RNAs in human mast cells and basophils by competitive reverse transcription-polymerase chain reaction. J Immunol 1995; 154:5472–5480.
26. Li L, Li Y, Reddel SW, Cherrian M, Friend DS, Stevens RL, Krilis SA. Identification of basophilic cells that express mast cell granule proteases in the peripheral blood of asthma, allergy, and drug-reactive patients. J Immunol 1998; 161:5079–5086.
27. Naclerio RM, Proud D, Togias AG, Adkinson NF Jr, Meyers DA, Kagey-Sobotka A, Plaut M, Norman PS, Lichtenstein LM. Inflammatory mediators in late antigen-induced rhinitis. N Engl J Med 1985; 313:65–70.
28. Mossman TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T-cell clones. I. Definition according to profiles of lymphokine activities and secreted protein. J Immunol 1986; 136:2348–2357.
29. Schroeder J, Kagey-Sobotka A, Lichtenstein L. The role of the basophil in allergic inflammation. Allergy 1995; 50:463–452.
30. MacGlashan DW Jr., White JM, Huang SK, Ono SJ, Schroeder J, Lichtenstein LM. Secretion of interleukin-4 from human basophils: the relationship between IL-4 mRNA and protein in resting and stimulated basophils. J Immunol 1994; 152:3006–3016.
31. Brunner T, Heusser CH, Dahinden CA. Human peripheral blood basophils primed by interleukin-3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. J Exp Med 1993; 177:605–611.
32. Gibbs B, Haas H, Falcone F, Albrecht C, Vollrath I, Noll T, Wolff H, Amon U. Purified human peripheral blood basophils release interleukin-13 and preformed interleukin-4 following immunological activation. Eur J Immunol 1996; 26:2493–2498.
33. Li H, Sim T, Alam R. IL-13 released by and localized in human basophils. J Immunol 1996; 156:4833–4838.
34. Kasaian M, Clay M, Happ M, Garman R, Hirani S, Luqman M. IL-4 production by allergen-stimulated primary cultures: identification of basophils as the major IL-4-producing cell type. Int Immunol 1996; 8:1287–1297.
35. Schroeder J, Howard B, Jenkens M, Kagey-Sobotka A, Lichtenstein L, MacGlashan DW Jr. IL-4 secretion and histamine release by human basophils are differentially regulated by protein kinase C activation. J Leuk Biol 1998; 63:692–698.
36. Prussin C, Foster B, Ludy LM, Metcalfe DD. Basophils are the predominant IL-4-producing peripheral blood cells in vivo and in vitro. J Allergy Clin Immunol 1998; 101:A177.
37. Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, Bews PA, Hunt TC, Okayama Y, Heusser CH, Bullock GR, Church MK, Holgate ST. Interleukin 4 is localized to and released by human mast cells. J Exp Med 1992; 176:1381–1386.
38. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, Heus-

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ser CH, Howart PH, Holgate ST. Interleukin-4,-5,-6 and tumour necrosis factor-a in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. Am J Respir Cell Mol Biol 1994; 10:471–480.
39. Jaffe J, Glaum M, Raible D, Post T, Dimitry E, Govindarao D, Wang Y, Schulman E. Human lung mast cell IL-5 gene and protein expression: temporal analysis of upregulation following IgE-mediated activation. Am J Respir Cell Mol Biol 1995; 13:665–675.
40. Pawanker R, Okuda M, Yssel H, Okumura K, Ra C. Nasal mast cells in perennial allergic rhinitics exhibit increased expression of the FcεRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. J Clin Invest 1997; 99:1492–1499.
41. Okayama Y, Petit-Frére C, Kassel O, Semper A, Quint D, Tunon-de-Lara MJ, Bradding P, Holgate ST, Church MK. IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells. J Immunol 1995; 155:1796–1808.
42. Gibbs BF, Arm JP, Gibson K, Lee TH, Pearce FL. Human lung mast cells release small amounts of interleukin-4 and tumour necrosis factor-alpha in response to stimulation by anti-IgE and stem cell factor. Eur J Pharmacol 1997; 327:73–78.
43. Ebisawa M, Shichijo M, Miura K, Tachimoto H, Katsunuma T, Onda T, Iikura Y, Saito H. Human mast cells produce TNF-α, MIP-1α and IL-5 following activation through FcεRI. J Allergy Clin Immunol 1995; 95:219A.
44. Vercelli D, Geha R. Regulation of IgE synthesis: from the membrane to the genes. Springer Semin Immunopathol 1993; 15:5–16.
45. de Vries JE, Zurawski G. Immunoregulatory properties of IL-13: its potential role in atopic disease. Int Arch Allergy Immunol 1995; 106:175–179.
46. Moser R, Fehr J. IL-4 controls the selective endothelium-driven transmigration of eosinophils from allergic individuals. J Immunol 1992; 149:1432–1438.
47. Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, Newman W, Luscinskas FW, Gimbrone MA, Bochner BS. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. J Immunol 1992; 148:1086–1092.
48. Bochner B, Klunk D, Sterbinsky S, Coffman R, Schleimer R. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. J Immunol 1995; 154:799–803.
49. Abehsira-Amar O, Gilbert M, Jolly M, Theze J, Jankovic D. IL-4 plays a dominant role in the differential development of Th0 into Th1 and Th2 cells. J Immunol 1992; 148:3820–3829.
50. Swain S, Weinberg A, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. J Immunol 1990; 145:3796–3806.
51. Gauchat J-F, Henchoz S, Mazzel G, Aubry J-P, Brunner T, Blasey H, Life P, Talabot D, Flores-Romo L, Thompson J, Kishl K, Butterfield J, Dahinden C, Bonnefoy J-Y. Induction of human IgE synthesis in B cells by mast cells and basophils. Nature 1993; 365:340–343.
52. Yanagihara Y, Kajiwara K, Basaki Y, Ikizawa K, Ebisawa M, Ra C, Tachimoto H, Saito H. Cultured basophils but not cultured mast cells induce IgE synthesis in B cells after immunologic stimulation. Clin Exp Immunol 1998; 111:136–143.
53. MacGlashan DW Jr., Peters SP, Warner J, Lichtenstein LM. Characteristics of human basophil sulfidopeptide leukotriene release: releasability defined as the

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270 Madison Avenue, New York, New York 10016

ability of the basophil to respond to dimeric cross-links. J Immunol 1986; 136: 2231–2239.
54. MacGlashan DW Jr. Releasability of human basophils: Cellular sensitivity and maximal histamine release are independent variables. J Allergy Clin Immunol 1993; 91:605–615.
55. Dembo M, Goldstein B. Theory of equilibrium binding of symmetric bivalent haptens to cell surface antibody: application to histamine release from basophils. J Immunol 1978; 121:345–353.
56. MacGlashan DW Jr., Dembo M, Goldstein B. Test of a theory relating to the cross-linking of IgE antibody on the surface of human basophils. J Immunol 1985; 135: 4129–4134.
57. Goldstein B, Perelson AS. Equilibrium theory for the clustering of bivalent cell surface receptors by trivalent ligands. Application to histamine release from basophils. Biophys J 1984; 45:1109–1123.
58. Wofsy C, Goldstein B. Cross-linking of Fcγ receptors and surface antibodies–theory and application. J Immunol 1990; 145:1814–1825.
59. Macken CA, Perelson AS. Aggregation of cell surface receptors by multivalent ligands. J Math Biol 1982; 14:365–370.
60. MacGlashan DW Jr., Bochner B, Adelman D, Jardieu P, Togias A, White J, Sterbinsky S, Hamilton R, Lichtenstein L. Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol 1997; 158:1438–1445.
61. Undem BJ, Pickett WC, Adams GK. Antigen-induced sulfidopeptide leukotriene release from the guinea pig superfused trachea Eur J Pharmacol 1987; 142:31–37.
62. MacGlashan DW Jr., Schleimer RP, Peters SP, Schulman ES, Adams GK, Sobotka AK, Newball HH, Lichtenstein LM. Comparative studies of human basophils and mast cells. Fed Proc 1983; 42:2504–2509.
63. Malveaux FJ, Conroy MC, Adkinson NF Jr., Lichtenstein LM. IgE receptors on human basophils: relationship to serum IgE concentration. J Clin Invest 1978; 62: 176–181.
64. Hsu C, MacGlashan JDW. IgE Antibody up-regulates high affinity IgE binding on murine bone marrow derived mast cells. Immunol Lett 1996; 52:129–134.
65. MacGlashan DW Jr., White J, Chichester K, Bochner BS, Davis FM, Schroeder JT, Lichtenstein LM. In vitro regulation of FcεRIα expression on human basophils by IgE antibody. Blood 1998; 91:1633–1643.
66. Lantz CS, Yamaguchi M, Oettgen HC, Katona IM, Miyajama I, Kinet JP, Galli SJ. IgE regulates mouse basophil FcεRI expression in vivo. J Immunol 1997; 158: 2517–2521.
67. Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajama I, Kinet JP, Galli SJ. IgE enhances mouse mast cell FcεRI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. J Exp Med 1997; 185:663–672.
68. Saini SS, MacGlashan DW Jr., Sterbinsky SA, Togias AK, Adelman DC, Lichtenstein LM, Bochner BS. Downregulation of human basophil IgE and FcεRIα surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo. J Immunol 1999; 162:5624–5630.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

69. MacGlashan DW Jr., Lichtenstein LM, McKenzie-White J, Chichester K, Henry AJ, Sutton BJ, Gould HJ. Upregulation of FcεRI on human basophils by IgE antibody is mediated by interaction of IgE with FcεRI. J Allergy Clin Immunol 1999; 104:492–498.
70. Isersky C, Rivera J, Mims S, Triche TJ. The fate of IgE bound to rat basophilic leukemia cells. J Immunol 1979; 122:1926–1936.
71. Furuichi K, Rivera J, Buonocore LM, Isersky C. Recycling of receptor-bound IgE by rat basophilic leukemia cells. J Immunol 1986; 136:1015–1022.
72. Ryan JJ, DeSimone S, Klisch G, Shelburne C, McReynolds LJ, Han K, Kovacs R, Mirmonsef P, Huff TF. IL-4 inhibits mouse mast cell FcεRI expression through a STAT6-dependent mechanism J Immunol 1998; 161:6915–6923.
73. Xia HZ, Du Z, Craig S, Klisch G, Noben-Trauth N, Kochan JP, Huff TH, Irani AM, Schwartz LB. Effect of recombinant human IL-4 on tryptase, chymase, and FcεRI expression in recombinant human stem cell factor- dependent fetal liver-derived human mast cells. J Immunol 1997; 159:2911–2921.
74. Yamaguchi M, Sayama K, Yano K, Lantz CS, Noben-Trauth N, Ra C, Costa JJ, Galli SJ. IgE enhances FcεRI expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood- derived mast cells: synergistic effect of IL-4 and IgE on human mast cell FcεRI expression and mediator release. J Immunol 1999; 162:5455–5465.
75. Thompson HL, Metcalfe DD, Kinet JP. Early expression of high-affinity receptor for immunoglobulin E (FcεRI) during differentiation of mouse mast cells and human basophils J Clin Invest 1990; 85:1227–1233.
76. Pang J, Taylor GR, Munroe DG, Ishaque A, Fung-Leung W-P, Lau CY, Liu F-T, Zhou L. Characterization of the gene for the human high affinity IgE receptor (FcεRI) alpha-chain J Immunol 1993; 151:6166–6174.
77. Nishiyama C, Yokota T, Okumura K, Ra C. The transcription factors elf-1 and GATA-1 bind to cell-specific enhancer elements of human high-affinity IgE receptor alpha-chain gene. J Immunol 1999; 163:623–630.
78. Zon LI, Gurish MF, Stevens RL, Mather C, Reynolds DS, Austen KF, Orkin SH. GATA-binding transcription factors in mast cells regulate the promoter of the mast cell carboxypeptidase A gene. J Biol Chem 1991; 266:22948–22953.
79. Blank U, Ra C, Miller L, White K, Metzger H, Kinet JP. Complete structure and expression in transfected cells of high affinity IgE receptor. Nature 1989; 337:187–189.
80. Miller L, Blank U, Metzger H, Kinet J. Expression of high-affinity binding of human IgE by transfected cells. Science 1989; 244:334–337.
81. Ra C, Jouvin MH, Kinet JP. Complete structure of the mouse mast cell receptor for IgE (FcεRI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. J Biol Chem 1989; 264:15323–15327.
82. Kuster H, Thompson H, Kinet JP. Characterization and expression of the gene for the human Fc receptor gamma subunit. Definition of a new gene family. J Biol Chem 1990; 265:6448–6452.
83. Lin S, Cicaia C, Scharenberg AM, Kinet JP. The FcεRIβ subunit functions as an amplifier of FcεRIγ-mediated cell activation signals. Cell 1996; 85:985–995.
84. Sihra B, Kon O, Grant J, Kay A. Expression of high-affinity IgE receptors (FcεRI)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

on peripheral blood basophils, monocytes, and eosinophils in atopic subjects: relationship to total serum IgE concentrations. J Allergy Clin Immunol 1997; 99:699–706.
85. Saini S, MacGlashan DW Jr., Klion A, Holland S, Hamilton R, Bochner B. Relationship between serum IgE and FceRI levels on human basophils, monocytes, and eosinophils. J Allergy Clin Immunol 1999; 103:S168.
86. Maurer D, Feiberger E, Reininger B, Wolf-Winiski B, Jouvin MH. Expression of functional high affinity immunoglobulin E receptors (FcεRI) on monocytes of atopic individuals. J Exp Med 1994; 179:745–750.
87. Reischl IG, Corvaia F, Effenberger F, Wolf-Winiski B, Kromer E, Mudde GC. Function and regulation of FcεRI expression on monocytes from non-atopic donors. Clin Exp Allergy 1995; 26:630–641.
88. Maurer D, Ebner C, Reininger B, Fieberger E, Kraft D, Kinet JP, Stingl G. The high affinity IgE receptor (FcεRI) mediates IgE-dependent allergen presentation. J Immunol 1995; 154:6285–6290.
89. Takenada M, Tanaka Y, Yoshida H, Ra C. High affinity IgE receptor-mediated prostaglandin E2 production by monocytes in atopic dermatitis. Int Arch Allergy Immunol 1995; 108:247–253.
90. Gounni AS, Lamhkhioued B, Ochial K, Tanaka Y, Delaporte E, Capron A, Kinet JP, Capron M. High-affinity IgE receptor on eosinophils is involved in defence against parasites. Nature 1994; 367:183–186.
91. Kita H, Gleich GJ. Eosinophils and IgE receptors: a continuing controversy. Blood 1997; 89:3497–3501.
92. Seminario MC, Saini SS, MacGlashan D Jr., Bochner BS. Intracellular expression and release of FcεRIα by human eosinophils. J Immunol 1999; 162:6893–6900.
93. Terada N, Konno Y, Terada S, Fukuda T, Yamashita T. IL-4 upregulates FcεRIα-chain messenger RNA in eosinophils. J Allergy Clin Immunol 1996; 96:1161–1169.
94. Rajakulasingam K, Durham S, O'Brien F, Humbert M, Barata L, Reece L, Kay A, Grant J. Enhanced expression of high-affinity IgE receptor (FcεRI) α chain in human allergen-induced rhinitis with co-localization to mast cells, macrophages, eosinophils, and dendritic cells. J Allergy Clin Immunol 1997; 100:78–86.
95. Ying S, Barata L, Meng Q, Grant JA, Barkans J, Durham S, Kay A. High-affinity immunoglobulin E receptor (FcεRI)-bearing eosinophils, mast cells, macrophages and Langerhans cells in allergen-induced late-phase cutaneous reactions in atopic subjects. Immunology 1998; 93:281–288.
96. Kita H, Kaneko M, Bartemes KR, Weiler D, Schimming AW, Reed CE, Gleich GJ. Does IgE bind to and activate eosinophils from patients with allergy? J Immunol 1999; 162:6901–6911.
97. Wang B, Rieger A, Kilgus O, Ochiai K, Maurer D, Fodinger D, Kinet J-P, Stingl G. Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. J Exp Med 1992; 175:1353–1365.
98. Jurgens M, Wollenberg A, Hanau D, de la Salle H, Bieber T. Activation of human epidermal Langerhans cells by engagement of the high affinity receptor for IgE, FcεRI. J Immunol 1995; 155:5184–5189.
99. Kraft S, WeBendorf JHM, Hanau D, Bieber T. Regulation of the high affinity recep-

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

tor for IgE on human epidermal Langerhans cells. J Immunol 1998; 161:1000–1006.
100. Maurer D, Fiebiger E, Ebner C, Reininger B, Fischer G, Wichlas S, Jouvin M, Schmitt-Egenolf M, Kraft D, Kinet J, Stingl G. Peripheral blood dendritic cells express FcεRI as a complex composed of FcεRIα- and FcεRIγ-chains and can use this receptor for IgE-mediated allergen presentation. J Immunol 1996; 157:607–616.
101. Joseph M, Gounni A, Kusnierz J, Vorng H, Sarfati M, Kinet J, Tonnel A, Capron A, Capron M. Expression and function of the high-affinity IgE receptor on human platelets and megakaryocyte precursors. Eur J Immunol 1997; 27:2212–2218.
102. Hasegawa S, Pawankar R, Suzuki K, Nakahata T, Furukawa S, Okumura K, Ra C. Functional expression of the high affinity receptor for IgE (FcεRI) in human platelets and its intracellular expression in human megakaryocytes. Blood 1999; 93:2543–2551.
103. Shields RL, Whether WR, Zioncheck K, O'Connell L, Fendly B, Presta LG, Thomas D, Saban R, Jardieu P. Inhibition of allergic reactions with antibodies to IgE. Int Arch Allergy Immunol 1995; 107:308–312.
104. Haak-Frendscho M, Robbins K, Lyons R, Shields R, Hooley J, Schoenhoff M, Jardieu P. Administration of an anti-IgE antibody inhibits CD23 expression and IgE production in vivo. Immunology 1994; 82:306–313.
105. Boulet L, Chapman K, Cote J, Kalra S, Bhagat R. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. Am J Respir Crit Care Med 1997; 155:1835–1840.
106. Fahy J, Fleming H, Wong H, Liu J, Su J, Reiman J, Fick R, Boushey H. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med 1997; 155: 1828–1834.
107. Togias A, Corren J, Shapiro G, Reimann A, von Schlegell A, Wighton T, Adelman D. Anti-IgE treatment reduces skin test reactivity. J Allergy Clin Immunol 1998; 101:S171.
108. Corne J, Djukanovic R, Thomas L, Warner J, Botta L, Grandordy B, Gygax D, Heusser C, Patalano F, Richardson W, Kilchherr E, Staehelin T, Davis F, Gordon W, Sun L, Liou R, Wang G, Chang TW, Holgate S. The effect of intravenous administration of a chimeric anti-IgE antibody on serum IgE levels in atopic subjects-efficacy, safety, and pharmacokinetics. J Clin Invest 1997; 99:879–887.
109. Heusser C, Jardieu P. Therapeutic potential of anti-IgE antibodies. Curr Opin Immunol 1997; 9:805–813.
110. Casale TB, Bernstein IL, Busse WW, LaForce CF, Tinkelman DG, Stoltz RR, Dockhorn RJ, Reimann J, Su JQ, Fick RB, Adelman DC. Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. J Allergy Clin Immunol 1997; 100:110–121.

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Expression of IgE Receptors on Eosinophils

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I. Introduction

IgE receptors bind either to the Fc portion of the immunoglobulin, and thus belong to the Fc receptor superfamily, or to carbohydrate moieties. The high-affinity receptor (FcεRI) and the low-affinity receptor (FcεRII/CD23) fall in the first group, while, Mac2/galectin-3, a member of the S-type lectin family, falls in the second one.

A high-affinity IgE receptor was initially described as expressed only on mast cells and basophils. Capron et al. (1) and Lawrence et al. (2), later reported on the presence of IgE receptors on rat macrophages and human lymphocytes, respectively. Indeed, a low-affinity IgE receptor belonging to the lectin superfamily, FcεRII/CD23, was then identified on various cell populations, in particular on B lymphocytes. FcεRI have been identified on various cell types, including mononuclear phagocytes (3), eosinophils (4), antigen-presenting cells (5–7), and platelets (8).

Over the last 8 years, several important findings, mainly due to the new experimental approaches involving molecular biology and genetically engineered animals, have modified our concepts of the role of eosinophils in immune re-

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sponse as well as the properties of FcεR. The present chapter will focus mainly on the expression and functions of the two IgE Fc receptors by eosinophils.

II. General Structure of FcεRI and FcεRII

Since an extensive review about FcεRI has been recently published (9), we encourage its reading for detailed information and references about the molecular structure of the receptor and the mechanisms of FcεRI-mediated signal transduction.

FcεRI is typically a tetrameric structure formed from one α chain (FcεRIα), one β chain (FcRβ), and a dimer of disulfide-linked γ chain (FcRγ). FcεRIα, the IgE-binding subunit of the receptor, is a glycoprotein belonging to the immunoglobulin superfamily and comprises two immunoglobulin-related domains (forming a highly bent structure), one transmembrane domain, and a short cytoplasmic tail. Crystal structure of the soluble form of FcεRIα, bound to the Fc fragment of an IgE molecule has been recently obtained (10,11). FcεRIα (even in its truncated soluble form) binds IgE with a 1:1 stoichiometry through it second immunoglobulin domain, although some residues in the first domain might contribute to the binding. The binding site was located in the Cε3 portion of the immunoglobulin. Binding affinity is 10^{-9}–10^{-10} M. FcRβ possess four transmembrane domains, intracytoplasmic amino- and carboxy-termini, shared, in mast cells, by both FcεRI and FcγRIII (CD16), a low-affinity IgG receptor. Polymorphisms in FcRβ have been associated to atopy. FcRγ chain is closely related to ζ and η chains. These subunits, composed of a transmembrane domain and a cytoplasmic tail, are found associated as homo- or heterodimers within several antigen receptors: FcαR (IgA receptor), FcεRI, FcγRI (high-affinity IgG receptor), FcγRIII, and T-cell receptor (TCR).

Both FcRβ and FcRγ are involved in signal transduction. They both possess an ITAM motif, which allows them to bind SH2 domains, present in nonreceptor tyrosine kinase. Upon clustering of receptor-bound IgE by multivalent antigen, Lyn kinase, which is in part bound to FcRβ independently of the ITAM motif, phosphorylates FcRβ and FcRγ ITAMS. This allows recruitment of additional Lyn by FcRβ and of Syk kinase by FcRγ. Then Lyn phosphorylates Syk, which in turn autophosphorylates. This leads to phosphorylation of downstream substrates by activated Syk.

The receptor expressed by mast cells and basophils is essentially an $\alpha\beta\gamma_2$ tetramer. In humans and in rat, but not in mouse, other cell types such as eosinophils, monocytes (3), dendritic cells (6), and Langerhans cells express a trimeric receptor lacking FcRβ, although FcRβ mRNA was detected in Langerhans cell from atopic patients (7) as well as in eosinophils (4). In the absence of FcRβ, membrane-bound Lyn phosphorylates FcRγ ITAM and Syk at a very low level.

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Indeed, it has been demonstrated that FcRβ was acting in vitro (12) and in vivo (13) as an amplifier of FcRγ-mediated cellular activation. Besides its role in signal transduction, FcRβ also promotes membrane expression of the receptor complexes by improving intracellular trafficking and processing of the heavily glycosylated FcεRIα (14). The combined amplification gain brought by the presence of FcRβ within the receptor has been estimated at between 12- and 30-fold (14). Thus, the various cell types expressing a trimeric FcεRI are much less efficient than mast cells and basophils, expressing a tetrameric receptor, in the translation of IgE-dependent triggering into biological effect.

The low-affinity receptor for IgE was shown to be identical to CD23, a molecule discovered independently on human lymphoblastoid B cells, on Epstein-Barr virus–transformed B cells, and as a B-cell activation antigen (reviewed in Ref. 15). FcεRII/CD23 is the only FcR that does not belong to the immunoglobulin gene superfamily. FcεRII/CD23 is a single copy gene with two isoforms, CD23a and b, produced by both alternative splicing and using different initiation sites for translation. They only differ by a short amino acid stretch in their intracytoplasmic region. FcεRII/CD23 is a type II membrane glycoprotein of 45 kDa, with a long C-terminal extracellular region, a single transmembrane domain, and a short N-terminal intracytoplasmic tail. The lectin domain, homologous to C-type lectins, contains the IgE-binding site (15). IgE binding to FcεRII/CD23 is calcium-dependent. A triplet of amino acids (Asp, Gly, Arg) is present at the carboxy-terminus of human FcεRII/CD23, but not in mouse CD23. This motif, in reverse orientation, is known as an adhesion sequence and is found in integrin receptors. Surface CD23 undergoes natural proteolytic cleavage to yield soluble fragments of 37 to 16 kDa, likely the result of an autocatalytic process. The presence of IgE reduces the rate of extracellular cleavage, whereas a fraction of CD23 can be internalized and intracellularly processed to a 16 kDa fragment by endocytosis (15).

The cellular distribution of the two isoforms, CD23a and CD23b, appeared different: FcεRII/CD23a is constitutively expressed on resting B cells, whereas FcεRII/CD23b is found on inflammatory cells as well as on IL-4–treated B cells. While CD23 expressed on B cells regulates antigen presentation to T cells, B-cell proliferation and differentiation into IgE-producing cells, FcεRII/CD23 on macrophages, eosinophils, and platelets mediates IgE-dependent cytotoxicity towards schistosome larvae in vitro (16) and thus could play a role in IgE-mediated protective immunity against helminth infections. A recent study has suggested a regulatory role for the region of chromosome 19, encompassing CD23 in atopic disorders. However, no polymorphism was detected in the coding sequence of CD23 (17).

Signal transduction through surface CD23 ligation is linked to cyclic nucleotides and nitric oxide (NO) pathways in various human cells and in rat macrophages (18). Some divergences are found between the signaling pathways cou-

pled to CD23a and CD23b. In contrast to CD23a expressed by B cells, crosslinking of CD23b on other human cells has little, if any, effect on Ca^{2+} influx and phosphoinositide pathway (18). These results, associated with the very short length of the intracytoplasmic domain of CD23, suggest that CD23 may be associated with other molecule(s) involved in signal transduction (18).

The soluble form of CD23 can also exert pleiotropic activities, which are independent of IgE binding. In particular, it has been shown that soluble CD23 could bind to CD21, the Epstein-Barr virus and complement receptor 2 (15), and to CD11b and CD11c integrins. Altogether, these results indicate that CD23 should not be viewed only as a low-affinity IgE receptor, but also as an adhesion molecule involved in cell-cell interactions. Alteration in CD23 balance could thus potentially lead to various diseases such as allergies and other inflammatory diseases.

III. Presence of IgE Receptors on Eosinophils

IgE receptors were initially detected on human eosinophils by binding assays such as IgE rosette formation, binding of radiolabeled IgE, and indirect flow cytometry (reviewed in Ref. 19). All these assays revealed that the expression of IgE receptors was restricted to a fraction of blood eosinophils, usually around 30%, a percentage of which was significantly increased when eosinophils were purified from hypereosinophilic patients. They also showed some heterogeneity in the binding of different IgE obtained from myeloma to eosinophils, a finding likely to explain the discrepancies between the results obtained by different groups (20). Nevertheless, evidence that IgE was binding in vivo to human eosinophils from eosinophilic patients with high IgE levels was provided in 1985. Indeed, cytophilically bound IgE was detected on tissue eosinophils and, to a lesser extent, to blood eosinophils (21). Moreover, treatment at acid pH of eosinophils from patients with parasitic infections led to elution of IgE molecules displaying antibody specificity, confirming the existence of cytophilically bound IgE antibodies to eosinophils from patients (19).

The existence of a saturable binding site for IgE on eosinophils was demonstrated by Scatchard analysis using radiolabeled IgE (22). The binding constant determined for monomeric IgE was in the range of 10^{-7} M^{-1}, which corresponds to a low affinity, similar to the affinity generally reported for the low-affinity IgG receptors (23). A saturable binding was detected only for eosinophils with low density (''hypodense'') but not for eosinophils with normal density (''normodense''). However, it must be pointed out that the low equilibrium constant associated with the heterogeneity of eosinophil populations could have limited the precision of such results. Since only an average of 30% eosinophils displays IgE

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binding, it is likely that the affinity constant would increase when taking into account only receptor-bearing populations.

Expression of IgE receptors on eosinophils has been also studied in rodents, in particular in the context of helminth infections. In the rat, presence of IgE receptors involved in protective immunity towards *Schistosoma mansoni*, a trematode parasite and causal agent of schistosomiasis, was demonstrated both in vitro and in vivo (24). Indeed, rat eosinophils incubated with serum from *Schistosoma*-infected animals containing anti-*Schistosoma* IgE or with a rat IgE monoclonal antibody towards *Schistosoma* secretion products displayed antibody-dependent cell-mediated cytotoxicity (ADCC) activity towards schistosomula in vitro, whereas incubation of eosinophils with an irrelevant IgE was ineffective (25,26). Furthermore, passive transfer of eosinophils from *Schistosoma*-infected animals (displaying high concentration of anti-*Schistosoma* IgE in their serum) to naive rats provided them with a significant level of protection during a challenge infection, while transfer of eosinophils from naïve animals was ineffective (22).

In the mouse, in striking contrast with both the human and the rat situations, the absence of FcεR, even on eosinophils present in granuloma from *Schistosoma*-infected animals, was unambiguously demonstrated (27).

A. Galectin-3

Galectin-3 (MAC-2/ε-BP) was first identified on macrophages (28), then on mast cells (29) and neutrophils (30). Expression of galectin-3 (MAC-2/ε-BP) was also detected at the surface of human eosinophils (31). An inhibition of IgE-dependent eosinophil-mediated cytotoxicity towards parasite targets by anti-Mac-2 mAb was observed, reflecting a functional role of this IgE binding structure.

Galectin-3 mRNA was detected in mouse eosinophils (27). Disruption of galectin-3 gene in mice has been shown to reduce macrophage-mediated peritoneal inflammation and seems to be involved in chemoattraction of monocytes and macrophages (32).

B. Identification of FcεRII/CD23 on Eosinophils

The inhibitory effects of both polyclonal and monoclonal antibodies cross-reacting with FcεRII/CD23 on the IgE-mediated functions of human eosinophils led us to suggest that eosinophils could express this receptor (33). Common biochemical characteristics were found between eosinophil FcεRII and the B-cell CD23 antigen. In particular, three polypeptides of 45–50, 23, and 15 kDa were detected by immunosorbent chromatography or by immunoprecipitation with a monoclonal antibody (mAb) directed to eosinophil IgE receptor (named BB10) or with IgE itself (33). Similarly, mAb 135 (anti-B-cell CD23) bound to a protein fraction immunoprecipitated by BB10 and reciprocally, on extracts from both

eosinophils and B cells, suggests the existence of cross-reacting molecules between eosinophil FcεRII and B-cell CD23 (33). Another similarity between eosinophil FcεRII and CD23 was the presence, in both structures, of an adhesion sequence. Detection of such a motif was performed by the binding of an antifibronectin antibody (Ab) in the case of eosinophils. It was shown to be present in the CD23 sequence with a reverse orientation. Similarly to B-cell CD23, the 45–50 kDa molecules immunoprecipitated from eosinophils might correspond to the membrane receptor, whereas the smaller molecular weight components could represent cleavage products, also released as soluble form in eosinophil supernatants and in blood of eosinophilic patients (34). Using flow cytometry and different anti-CD23 mAbs, a low expression of CD23 was observed on human eosinophils, with a large heterogeneity according to the patients (35). In a more recent study, performed by in situ hybridization and RT-PCR analysis, we demonstrated in eosinophils the presence of CD23 mRNA, totally homologous in sequence with B-cell CD23 (36). Moreover, both CD23a and CD23b isoforms were detected on eosinophils. These results led us to conclude that human eosinophils could synthesize and express the CD23 molecule, homologous to B-cell CD23 and involved in the IgE-mediated function of eosinophils.

Likewise, in rat the first IgE receptor identified on eosinophils and likely to mediate ADCC reactions was CD23 (37). As mentioned earlier, mice do not express CD23 on eosinophils (27).

C. Detection of FcεRI on Eosinophils—A Controversial Issue?

The demonstration in 1992 that FcεRI could be expressed in humans on cell types other than mast cells and basophils, namely Langerhans cells from epidermis (7), raised the possibility that human eosinophils would also be able to express FcεRI, besides expressing CD23. Two years later it was indeed shown that highly purified eosinophils from patients with marked eosinophilia expressed a functional FcεRI (4). A mAb recognizing FcεRIα (clone 15-1) was used in flow cytometry to detect surface expression of the receptor. Expression was very heterogeneous among the different patients. FcεRI was detected not only on highly purified blood eosinophils but also on skin eosinophils from atopic dermatitis patients by immunostaining of skin biopsies with the 15-1 mAb (38). In addition, FcεRIα mRNA was detected by Northern blot, while the FcRβ and FcRγ mRNA were only detectable by RT-PCR. The anti-FcεRIα mAb was also able to inhibit binding of ^{125}I-IgE. Altogether, these results demonstrated that a functional FcεRI was expressed on human eosinophils. However, as observed for Langerhans cells and monocytes, expression levels are usually very low (and heterogeneous) when compared to mast cells and basophils. Because FcRβ protein has not been detected so far in human eosinophils, one can suspect that its absence can account for this low expression levels.

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These apparently clear-cut results are still challenged by some investigators. Indeed, in a recent study, the authors were unable to detect surface expression of the receptor on eosinophils from patients, while surprisingly evidencing a significant intracellular pool of FcεRIα as well as soluble receptor (39). Another report mentioned that eosinophils expressed about 0.5% of FcεRI expressed on basophils but could not be induced, upon receptor crosslinking with IgE and anti-IgE, to degranulate, to produce superoxide anions, or to release leukotriene C_4 (40). A third report mentioned that eosinophils from atopic patients were expressing negligible amounts of FcεRI and that EPO was not released upon receptor activation (41). Such findings are likely due to the lack of sensitivity of the detection method used and, at least for the last report, to the inadequate activation protocol (absence of crosslinking of receptor-bound anti-FcεRIα with a secondary antibody).

As we observed for CD23, the status of FcεRI on rat and mouse eosinophils is very different. Our recent results have demonstrated that, in rats, upon thioglycollate elicitation, peritoneal eosinophils expressed a functional trimeric $\alpha\gamma_2$ FcεRI able to mediate IgE-dependent ADCC towards schistosomula (42). In mouse, countless studies have reported on eosinophilia as a hallmark of pulmonary inflammation and of granuloma formation. Since wild-type mice do not express FcεR (27), this species does not appear to be the best suited model for the study of IgE-dependent, eosinophil-mediated reactions, as already mentioned by Jones et al. (43) after experiments on a model of parasitic infections.

In order to obtain an animal model that would reproduce the cellular distribution of FcεRI found in humans, transgenic (Tg) mice expressing the human FcεRα chain (hFcεRα) under the control of its own promoter elements were generated (13,44). In these animals, not only mast cells, but also eosinophils, macrophages, and Langerhans cells expressed a functional receptor, resulting from the association of hFcεRα with the endogenous FcRγ. Such murine cells with a humanized FcεRI able to bind both human and murine IgE represent a novel experimental model more relevant that the wild-type mouse models to evaluate the role of IgE-dependent effector mechanisms both in vitro and in vivo. Indeed, eosinophils from *Schistosoma mansoni*–infected transgenic animals displayed FcεRI-dependent ADCC towards parasitic larvae, whereas eosinophils from infected WT animals were not cytotoxic (D. Dombrowicz et al., unpublished observations). However, like their wild-type counterparts and unlike rats, naive hFcεRα Tg animals harbor a very small number of eosinophils, which only increases upon antigen sensitization or helminth infection. In order to obtain naive animals with high number of hFcεRI-positive eosinophils, hFcεRIα Tg mice were crossed with animals overexpressing IL-5 in T cells (using hCD2 promoter) (45). As expected, the resulting double transgenic mice displayed splenic, peritoneal, and medullar eosinophilia. Like in humans, eosinophils obtained from these animals were expressing FcεRI, but on a low proportion of eosinophils. Due to their

low granular content, eosinophils from naive animals (46) did not exhibit ADCC towards schistosomula. However, they displayed IgE- and FcεRI-mediated adherence to the larvae.

It thus appear that, from both morphological and functional points of view, rat eosinophils are much closer to human ones than their murine counterparts. Therefore, the results regarding the involvement of IgE and eosinophils in murine models of asthma are not necessarily relevant to the cognate human pathology.

D. Regulation of FcεRI Expression

FcεRI expression is regulated by at least two factors, namely IL-4 and IgE. A correlation was first observed between serum IgE levels and IgE receptors present on human basophils (47,48). These results were further extended by Sihra et al. (49), who showed that a correlation between IgE levels and FcεRI expression existed not only for basophils, but also for monocytes. Kita et al. (40), as well as our own group, were able to show such a correlation for eosinophils. However, two other groups did not observe the same phenomenon (39,49). Nevertheless, we were able to reproduce such an upregulation of FcεRI expression by IgE in vitro using eosinophils from either normal donors, thioglycollate-elicited rat peritoneal eosinophils, or splenic eosinophils from IL-5 × hFcεRIα Tg mice (D. Dombrowicz et al., unpublished). On the other hand, IL-4 was shown to upregulate FcεRI expression on human mast cells derived from cord blood (50,51) or fetal liver (52). Terada et al. showed that blood eosinophils from patients with allergic rhinitis had increased mRNA for FcεRIα upon treatment with IL-4, whereas no effect could be detected at the protein level (53).

It appears nevertheless that FcεRI expression on eosinophils is increased in several allergic and inflammatory disorders. Increased numbers of FcεRIα mRNA and FcεRIα-positive cells were detected in the nasal mucosa of patients with atopic rhinitis, after allergen challenge, with around 15% cells identified as eosinophils by double immunostaining (54). A significant proportion of eosinophils expressing FcεRI was also detected in the skin of atopic subjects after allergen challenge during the late phase reaction (55–57).

Finally, the expression levels of FcεRI vary not only according to the pathology, but also probably according to the maturation stage and or tissue localization of eosinophils, as suggested by the fact that peritoneal or splenic eosinophils from IL-5 × hFcεRIα Tg mice expressed more receptors at their surface than bone marrow or blood eosinophils (D. Dombrowicz et al., unpublished).

IV. Functions in the Immune Response

The expression of both FcεRI and CD23 by human eosinophils enables this cell type to act in two ways in the immune response: as an effector and as a regulatory

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cell. Indeed, IgE binding to eosinophils and subsequent receptor engagement by crosslinking by a multivalent antigen or by anti-IgE antibodies has been shown to induce the specific release of some, but not all, cytotoxic proteins (EPO, MBP, EDN) contained within the granules (4,19,58). This led us to suggest that the release of granule proteins and the underlying degranulation mechanisms could differ according to the type of Fc receptor engaged, as demonstrated upon IgE (19) and IgA (59) binding, and confirmed upon FcεRI crosslinking. Inflammatory mediators such as leukotrienes and superoxide anions are also produced upon IgE-dependent activation (60). These molecules have the potential of being both beneficial and detrimental to the host. On the one hand, it has been demonstrated that, in the course of IgE-mediated ADCC reactions towards helminths, granular proteins were cytotoxic for *Schistosoma* larvae in vitro (4,16,19,35). Human eosinophils, together with platelets and macrophages/monocytes, which also express both FcεRI and CD23, might thus participate to anti-helminthic immunity in vivo. EPO in the presence of hydrogen peroxide and bromide ions has been shown to possess bactericidal properties (61,62). On the other hand, the same granular molecules display potent pro-inflammatory properties and are therefore able to be harmful to the host itself. Indeed, EPO allows bromination of tyrosine residues from the host's own proteins and can thus be responsible for tissue damage (63,64). Bromotyrosine concentrations have been shown to be greatly increased in broncho-alveolar lavages from asthmatic patients upon in vivo antigenic challenge (64).

It is also possible that IgE receptor expression would provide eosinophils with a means to exert their antitumoral activity through IgE-mediated ADCC. Indeed, it has been shown that eosinophils sometimes play a role in IL-4 antitumoral activity (65) and that FcαR (66), FcεRI (67,68), and FcγR (69) also participate to antitumoral activity.

Eosinophils have been recently associated with inflammatory reactions occurring during skin allograft rejection (70). In one model of graft-versus-host disease, the reaction was lethal and associated to IgE production (71). An involvement of FcεRI in this process has not been demonstrated so far.

As demonstrated for mast cells, eosinophils, which also synthesize several cytokines, are able to secrete cytokines upon FcεRI engagement and therefore modulate the immune response. Indeed, it was first demonstrated that activation of eosinophils by IgE immune complexes induced IL-5 release (72). More recently, and similarly to IgA immune complexes (59), we have shown that FcεRI or CD23 crosslinking induced the selective release of IL-10 by eosinophils. These cells would thus be able to polarize the immune response towards a type 2 profile.

Since it has been shown that FcεRI, expressed by professional antigen-presenting cells such as human epidermal Langerhans cells (73), dendritic cells (6), and monocytes (74), was able to very efficiently mediate antigen capture, processing, and presentation, eosinophils might possibly fulfill the same function.

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Indeed this cell type expresses not only MHC-II (75), but also co-stimulatory molecules such as CD28, CD80, and CD86 (59,76 Woerly et al., 1999). This potentially allows them to develop the appropriate cellular contacts and to deliver the required signals for T-cell activation. The antigen-presenting function of eosinophils was demonstrated in a murine model of aerosol sensitization (76). However, since murine eosinophils do not express FcεR, these receptors cannot affect these cells directly.

Finally, besides *Schistosoma* infection, IgE and eosinophils have been associated with protective immunity against *Trichinella* and *Necator* parasites in vivo in rats (77) and in humans (78), respectively.

V. Potential Pitfalls of Anti-IgE Therapy in Patients with Parasitic Infections

Increased IgE production is, besides atopic manifestations (asthma, rhinitis, atopic dermatitis), proverbially associated with helminth infections. It is estimated that 3.5 billion people are infected with intestinal and other helminths, causing pathology in approximately 450 million cases. Although these parasites induce low mortality directly, they take their toll on human health by establishing long-term chronic infections and are responsible for a high morbidity. It is estimated that more than 50% of infected individuals have a mean serum IgE level above 1000 units/mL, which is comparable or even superior to those of found in allergic patients. Polyclonal type 2 activation might account for these high IgE levels.

Pioneering observations in vitro in various models of schistosomiasis and in vivo demonstration in rats of the protective role of IgE have shed new light on the potential role of IgE in immune defense against helminth parasites. In humans, epidemiological correlations arising from seven different studies in various parts of the world strongly suggest that IgE may be one of the keys to protective immunity in schistosomiasis (79–85). From a general standpoint, it is now agreed that helminths drive a strong type 2 immune response characterized by IgE antibodies, eosinophilia, and mast cell expansion. While the role of mast cells in protective response against helminths, apart from intestinal nematodes, remains elusive, there is accumulating evidence that IgE antibodies are involved in antibody-mediated immunity and that eosinophils are key effector cells in the killing of various helminth larvae (e.g., schistosomes, filarial worms, *Trichinella*, intestinal nematodes).

Although it has been shown in some experimental circumstances that IgE responses might be beneficial to the parasite and that anti-IgE treatment (86) could increase immunity, it should be stressed that most of these observations were made in the mouse, which, unlike rats and humans, does not express FcεRI on effector and antigen-presenting cells (eosinophils, macrophages, and dendritic

cells). During recent years, the demonstration of IgE receptor expression on inflammatory cells has slightly changed our perspective regarding the cellular mechanisms of both immune defense against helminths and allergic reactions. The IgE-dependent release of multiple inflammatory proteins by these cell populations allows them to participate in inflammatory processes associated with allergic reactions. Early experiments regarding the mechanisms of activation have revealed, in particular for macrophages and eosinophils, the key role played by IgE immune complexes.

All these considerations lead us to recommend the greatest care for immuno-intervention using anti-IgE antibodies (87–89) in areas with high rates of helminth infections. Besides the potential risk of decreasing immune defense mechanisms in individuals exposed to helminth infections, the massive production of IgE–anti-IgE complexes in individuals with high IgE levels could lead to the undesirable activation of inflammatory cells and the occurrence of deleterious events.

A final consideration regards the intriguing but so far unexplained lack of prevalence of allergic manifestations in countries characterized by high endemicity of helminth infections, namely tropical areas such as Africa, Central and South America, and some Asian countries (90). It is noticeable that the use of antihelminthic drugs has led to the emergence of allergic diseases in populations previously infected by helminths. This situation has been particularly striking in Japan and in some countries in South America where the disappearance of parasitic diseases following industrialization led to a dramatic increase in the prevalence of allergy. This raises the fascinating question of the actual role, during evolution, of the IgE molecule, originally targeted against helminth infections and now reacting against environmental allergens in industrial countries. It also calls into question strategies aiming at massive administration of anti-IgE antibodies in areas of endemic infections by helminths.

References

1. Capron A, Dessaint JP, Capron M, Bazin H. Specific IgE antibodies in immune adherence of normal macrophages to *Schistosoma mansoni* schistosomules. Nature 1975; 253:474–475.
2. Lawrence DA, Weigle WO, Spiegelberg HL. Immunoglobulins cytophilic for human lymphocytes, monocytes, and neutrophils. J Clin Invest 1975; 55:368–387.
3. Maurer D, Fiebiger E, Reininger B, Wolff-Winiski B, Jouvin MH, Kilgus O, Kinet JP, Stingl G. Expression of functional high affinity immunoglobulin E receptors (FcεRI) on monocytes of atopic individuals. J Exp Med 1994; 179:745–750.
4. Gounni AS, Lamkhioued B, Ochiai K, Tanaka Y, Delaporte E, Capron A, Kinet JP, Capron M. High-affinity IgE receptor on eosinophils is involved in defence against parasites. Nature 1994; 367:183–186.

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270 Madison Avenue, New York, New York 10016

5. Bieber T, de la Salle H, Wollenberg A, Hakimi J, Chizzonite R, Ring J, Hanau D, de la Salle C. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (FcεRI). J Exp Med 1992; 175:1285–1290.
6. Maurer D, Fiebiger S, Ebner C, Reininger B, Fischer GF, Wichlas S, Jouvin MH, Schmitt-Egenolf M, Kraft D, Kinet JP, Stingl G. Peripheral blood dendritic cells express FcεRI as a complex composed of FcεRI alpha- and FcεRI gamma-chains and can use this receptor for IgE-mediated allergen presentation. J Immunol 1996; 157:607–616.
7. Wang B, Rieger A, Kilgus O, Ochiai K, Maurer D, Fodinger D, Kinet JP, Stingl G. Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. J Exp Med 1992; 175:1353–1365.
8. Joseph M, Gounni AS, Kusnierz JP, Vorng H, Sarfati M, Kinet JP, Tonnel AB, Capron A, Capron M. Expression and functions of the high-affinity IgE receptor on human platelets and megakaryocyte precursors. Eur J Immunol 1997; 27:2212–2218.
9. Kinet JP. The high-affinity IgE receptor (FcεRI): from physiology to pathology. Annu Rev Immunol 1999; 17:931–972.
10. Garman SC, Kinet JP, Jardetzky TS. Crystal structure of the human high-affinity IgE receptor. Cell 1998; 95:951–961.
11. Garman SC, Wurzburg BA, Tarchevskaya SS, Kinet JP, Jardetzky TS. Structure of the Fc fragment of human IgE bound to its high-affinity receptor FcεRI alpha. Nature 2000; 406:259–266.
12. Lin S, Cicala C, Scharenberg AM, Kinet JP. The Fc(epsilon)RIbeta subunit functions as an amplifier of Fc(ε)RIgamma-mediated cell activation signals. Cell 1996; 85: 985–995.
13. Dombrowicz D, Lin S, Flamand V, Brini AT, Koller BH, Kinet JP. Allergy-associated FcRbeta is a molecular amplifier of IgE- and IgG-mediated in vivo responses. Immunity 1998; 8:517–529.
14. Donnadieu E, Jouvin MH, Kinet JP. A second amplifier function for the allergy-associated FcεRI-beta subunit. Immunity 2000; 12:515–523.
15. Bonnefoy JY, Lecoanet-Henchoz S, Gauchat JF, Graber P, Aubry JP, Jeannin P, Plater-Zyberk C. Structure and functions of CD23. Int Rev Immunol 1997; 16:113–128.
16. Capron A, Dessaint JP, Capron M, Joseph M, Ameisen JC, Tonnel AB. From parasite to allergy: the second receptor for IgE (FcεR2). Immunol Today 1986; 7:15–18.
17. Laitinen T, Ollikainen V, Lazaro C, Kauppi P, de Cid R, Anto JM, Estivill X, Lokki H, Mannila H, Laitinen LA, Kere J. Association study of the chromosomal region containing the FCεR2 gene suggests it has a regulatory role in atopic disorders. Am J Respir Crit Care Med 2000; 161:700–706.
18. Mossalayi MD, Arock M, Debre P. CD23/FcεRII: signaling and clinical implication. Int Rev Immunol 1997; 16:129–146.
19. Capron M, Grangette C, Torpier G, Capron A. The second receptor for IgE in eosinophil effector function. Chem Immunol 1989; 47:128–178.
20. Kita H, Gleich GJ. Eosinophils and IgE receptors: a continuing controversy. Blood 1997; 89:3497–3501.
21. Capron M, Kusnierz JP, Prin L, Spiegelberg HL, Ovlaque G, Gosset P, Tonnel AB,

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Capron A. Cytophilic IgE on human blood and tissue eosinophils: detection by flow microfluorometry. J Immunol 1985; 134:3013–3018.
22. Capron M, Spiegelberg HL, Prin L, Bennich H, Butterworth AE, Pierce RJ, Ouaissi MA, Capron A. Role of IgE receptors in effector function of human eosinophils. J Immunol 1984; 132:462–468.
23. Ravetch JV, Kinet JP. Fc receptors. Annu Rev Immunol 1991; 9:457–492.
24. Capron M, Capron A. Immunoglobulin E and effector cells in schistosomiasis. Science 1994; 264:1876–1877.
25. Capron M, Bazin H, Joseph M, Capron A. Evidence for IgE-dependent cytotoxicity by rat eosinophils, J Immunol 1981; 126:1764–1768.
26. Verwaerde C, Joseph M, Capron M, Pierce RJ, Damonneville M, Velge F, Auriault C, Capron A. Functional properties of a rat monoclonal IgE antibody specific for *Schistosoma mansoni*. J Immunol 1987; 138:4441–4446.
27. de Andres B, Rakasz E, Hagen M, McCormik ML, Mueller AL, Elliot D, Metwali A, Sandor M, Britigan BE, Weinstock JV, Lynch RG. Lack of Fcε receptors on murine eosinophils: implications for the functional significance of elevated IgE and eosinophils in parasitic infections. Blood 1997; 89:3826–3836.
28. Cherayil BJ, Chaitovitz S, Wong C, Pillai S. Molecular cloning of a human macrophage lectin specific for galactose. Proc Natl Acad Sci USA 1990; 87:7324–7328.
29. Frigeri LG, Liu FT. Surface expression of functional IgE binding protein, an endogenous lectin, on mast cells and macrophages, J Immunol 1992; 148:861–867.
30. Truong MJ, Gruart V, Kusnierz JP, Papin JP, Loiseau S, Capron A, Capron M. Human neutrophils express immunoglobulin E (IgE)-binding proteins (Mac-2/ε BP) of the S-type lectin family: role in IgE-dependent activation. J Exp Med 1993; 177: 243–248.
31. Truong MJ, Gruart V, Liu FT, Prin L, Capron A, Capron M. IgE-binding molecules (Mac-2/ε BP) expressed by human eosinophils. Implication in IgE-dependent eosinophil cytotoxicity. Eur J Immunol 1993; 23:3230–3235.
32. Hsu DK, Yang RY, Pan Z, Yu L, Salomon DR, Fung-Leung WP, Liu FT. Targeted disruption of the galectin-3 gene results in attenuated peritoneal inflammatory responses. Am J Pathol 2000; 156:1073–1083.
33. Grangette C, Gruart V, Ouaissi MA, Rizvi F, Delespesse G, Capron A, Capron M. IgE receptor on human eosinophils (FcεRII). Comparison with B cell CD23 and association with an adhesion molecule. J Immunol 1989; 143:3580–3588.
34. Aldebert D, Prin L, Dessaint JP, De Groote D, Capron A, Capron M. Elevation of soluble CD23 in serum from patients with blood eosinophilia. Int Arch Allergy Immunol 1994; 103:245–251.
35. Capron M, Truong MJ, Aldebert D, Gruart V, Suemura M, Delespesse G, Tourvieille B, Capron A. Heterogeneous expression of CD23 epitopes by eosinophils from patients. Relationships with IgE-mediated functions. Eur J Immunol 1991; 21:2423–2429.
36. Gounni AS, Lamkhioued B, Morita M, Aldebert D, Sarfati M, Capron A, Capron M. Molecular characterization of the low affinity IgE receptor FcεRII/CD23 expressed by human eosinophils. Int Immunol 1998; 10:395–404.
37. Capron M, Jouault T, Prin L, Joseph M, Ameisen JC, Butterworth AE, Papin JP, Kusnierz JP, Capron A. Functional study of a monoclonal antibody to IgE Fc recep-

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tor (FcεR2) of eosinophils, platelets, and macrophages. J Exp Med 1986; 164:72–89.
38. Gounni AS, Lamkhioued B, Delaporte E, Dubost A, Kinet JP, Capron A, Capron M. The high-affinity IgE receptor on eosinophils: from allergy to parasites or from parasites to allergy? J Allergy Clin Immunol 1994; 94:1214–1216.
39. Seminario MC, Saini SS, MacGlashan DW, Jr., Bochner BS. Intracellular expression and release of FcεRI alpha by human eosinophils. J Immunol 1999; 162:6893–6900.
40. Kita H, Kaneko M, Bartemes KR, Weiler DA, Schimming AW, Reed CE, Gleich GJ. Does IgE bind to and activate eosinophils from patients with allergy? J Immunol 1999; 162:6901–6911.
41. Smith SJ, Ying S, Meng Q, Sullivan MH, Barkans J, Kon OM, Sihra B, Larche M, Levi-Schaffer F, Kay AB. Blood eosinophils from atopic donors express messenger RNA for the alpha, beta, and gamma subunits of the high-affinity IgE receptor (FcεRI) and intracellular, but not cell surface, alpha subunit protein. J Allergy Clin Immunol 2000; 105:309–317.
42. Dombrowicz D, Quatannens B, Papin JP, Capron A, Capron M. Expression of a functional FcεRI on rat eosinophils and macrophages. J Immunol 2000; 165:1266–1271.
43. Jones RE, Finkelman FD, Hester RB, Kayes SG. *Toxocara canis*: failure to find IgE receptors (FcεR) on eosinophils from infected mice suggests that murine eosinophils do not kill helminth larvae by an IgE-dependent mechanism. Exp Parasitol 1994; 78:64–75.
44. Dombrowicz D, Brini AT, Flamand V, Hicks E, Snouwaert JN, Kinet JP, Koller BH. Anaphylaxis mediated through a humanized high affinity IgE receptor. J Immunol 1996; 157:1645–1651.
45. Dent LA, Strath M, Mellor AL, Sanderson CJ. Eosinophilia in transgenic mice expressing interleukin 5. J Exp Med 1990; 172:1425–1431.
46. Mahakunkijcharoen Y, Osada Y, Nara T, Horie T, Takatsu K, Kojima S. Comparative studies on schistosomulicidal activity of mouse and rat eosinophils. Int Arch Allergy Immunol 1997; 114(suppl 1):40–44.
47. Conroy MC, Adkinson NF, Jr., Lichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. J Immunol 1977; 118:1317–1321.
48. Malveaux FJ, Conroy MC, Adkinson NF, Jr., Lichtenstein LM. IgE receptors on human basophils. Relationship to serum IgE concentration. J Clin Invest 1978; 62: 176–181.
49. Sihra BS, Kon OM, Grant JA, Kay AB. Expression of high-affinity IgE receptors (FcεRI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. J Allergy Clin Immunol 1997; 99:699–706.
50. Toru H, Pawankar R, Ra C, Yata J, Nakahata T. Human mast cells produce IL-13 by high-affinity IgE receptor cross- linking: enhanced IL-13 production by IL-4-primed human mast cells. J Allergy Clin Immunol 1998; 102:491–502.
51. Yamaguchi M, Sayama K, Yano K, Lantz CS, Noben-Trauth N, Ra C, Costa JJ, Galli SJ. IgE enhances Fcε receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: syn-

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ergistic effect of IL-4 and IgE on human mast cell Fcε receptor I expression and mediator release. J Immunol 1999; 162:5455–5465.
52. Xia HZ, Du Z, Craig S, Klisch G, Noben-Trauth N, Kochan JP, Huff TH, Irani AM, Schwartz LB. Effect of recombinant human IL-4 on tryptase, chymase, and Fcε receptor type I expression in recombinant human stem cell factor- dependent fetal liver-derived human mast cells, J Immunol 1997; 159:2911–2921.
53. Terada N, Konno A, Terada Y, Fukuda S, Yamashita T, Abe T, Shimada H, Ishida K, Yoshimura K, Tanaka Y, et al. IL-4 upregulates FcεRI α-chain messenger RNA in eosinophils. J Allergy Clin Immunol 1995; 96:1161–1169.
54. Rajakulasingam K, Durham SR, O'Brien F, Humbert M, Barata LT, Reece L, Kay AB, Grant JA. Enhanced expression of high-affinity IgE receptor (FcεRI) alpha chain in human allergen-induced rhinitis with co-localization to mast cells, macrophages, eosinophils, and dendritic cells. J Allergy Clin Immunol 1997; 100:78–86.
55. Barata LT, Ying S, Grant JA, Humbert M, Barkans J, Meng Q, Durham SR, Kay AB. Allergen-induced recruitment of FcεRI+ eosinophils in human atopic skin. Eur J Immunol 1997; 27:1236–1241.
56. Tanaka Y, Takenaka M, Matsunaga Y, Okada S, Anan S, Yoshida H, Ra C. High affinity IgE receptor (FcεRI) expression on eosinophils infiltrating the lesions and mite patch tested sites in atopic dermatitis. Arch Dermatol Res 1995; 287:712–771.
57. Ying S, Barata LT, Meng Q, Grant JA, Barkans J, Durham SR, Kay AB. High-affinity immunoglobulin E receptor (FcεRI)-bearing eosinophils, mast cells, macrophages and Langerhans' cells in allergen-induced late-phase cutaneous reactions in atopic subjects. Immunology 1998; 93:281–288.
58. Tomassini M, Tsicopoulos A, Tai PC, Gruart V, Tonnel AB, Prin L, Capron A, Capron M. Release of granule proteins by eosinophils from allergic and nonallergic patients with eosinophilia on immunoglobulin-dependent activation. J Allergy Clin Immunol 1991; 88:365–375.
59. Woerly G, Roger N, Loiseau S, Dombrowicz D, Capron A, Capron M. Expression of CD28 and CD86 by Human Eosinophils and Role in the Secretion of Th1 (IL-2 and IFN-γ) cytokines. Inhibition by IgA complexes. J Exp Med 1999; 190:487–495.
60. Costa JJ, Weller PF, Galli SJ. The cells of the allergic response: mast cells, basophils, and eosinophils. JAMA 1997; 278:1815–1822.
61. Jong EC, Henderson WR, Klebanoff SJ. Bactericidal activity of eosinophil peroxidase. J Immunol 1980; 124:1378–1382.
62. Weiss SJ, Test ST, Eckmann CM, Roos D, Regiani S. Brominating oxidants generated by human eosinophils. Science 1986; 234:200–203.
63. Wu W, Chen Y, Hazen SL. Eosinophil peroxidase nitrates protein tyrosyl residues. Implications for oxidative damage by nitrating intermediates in eosinophilic inflammatory disorders. J Biol Chem 1999; 274:25933–25944.
64. Wu W, Samoszuk MK, Comhair SA, Thomassen MJ, Farver CF, Dweik RA, Kavuru MS, Erzurum SC, Hazen SL. Eosinophils generate brominating oxidants in allergen-induced asthma. J Clin Invest 2000; 105:1455–1463.
65. Tepper RI, Coffman RL, Leder P. An eosinophil-dependent mechanism for the antitumor effect of interleukin-4. Science 1992; 257:548–551.
66. Deo YM, Sundarapandiyan K, Keler T, Wallace PK, Graziano RF. Bispecific mole-

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cules directed to the Fc receptor for IgA (FcαRI, CD89) and tumor antigens efficiently promote cell-mediated cytotoxicity of tumor targets in whole blood. J Immunol 1998; 160:1677–1686.
67. Gould HJ, Mackay GA, Karagiannis SN, O'Toole CM, Marsh PJ, Daniel BE, Coney LR, Zurawski Jr, VR, Joseph M, Capron M, et al. Comparison of IgE and IgG antibody-dependent cytotoxicity in vitro and in a SCID mouse xenograft model of ovarian carcinoma. Eur J Immunol 1999; 29:3527–3537.
68. Luiten RM, Warnaar SO, Schuuman J, Pasmans SG, Latour S, Daeron M, Fleuren GJ, Litvinov SV. Chimeric immunoglobulin E reactive with tumor-associated antigen activates human FcεRI bearing cells. Hum Antibodies 1997; 8:169–180.
69. Clynes R, Takechi Y, Moroi Y, Houghton A, Ravetch JV. Fc receptors are required in passive and active immunity to melanoma. Proc Natl Acad Sci USA 1998; 95: 652–665.
70. Le Moine A, Flamand V, Demoor FX, Noel JC, Surquin M, Kiss R, Nahori MA, Pretolani M, Goldman M, Abramowicz D. Critical roles for IL-4, IL-5, and eosinophils in chronic skin allograft rejection. J Clin Invest 1999; 103:1659–1667.
71. Coudert JD, Foucras G, Demur C, Coureau C, Mazerolles C, Delsol G, Druet P, Guery JC. Lethal host-versus-graft disease and hypereosinophilia in the absence of MHC I-T-cell interactions. J Clin Invest 2000; 105:1125–1132.
72. Dubucquoi S, Desreumaux P, Janin A, Klein O, Goldman M, Tavemier J, Capron A, Capron M. Interleukin 5 synthesis by eosinophils: association with granules and immunoglobulin-dependent secretion. J Exp Med 1994; 179:703–708.
73. Maurer D, Stingl G. Immunoglobulin E-binding structures on antigen-presenting cells present in skin and blood. J Invest Dermatol 1995; 104:707–710.
74. Maurer D, Ebner C, Reininger B, Fiebiger E, Kraft D, Kinet JP, Stingl G. The high affinity IgE receptor (FcεRI) mediates IgE-dependent allergen presentation. J Immunol 1995; 154:6285–6290.
75. Del Pozo V, De Andres B, Martin E, Cardaba B, Femandez JC, Gallardo S, Tramon P, Leyva-Cobian F, Palomino P, Lahoz C. Eosinophil as antigen-presenting cell: activation of T cell clones and T cell hybridoma by eosinophils after antigen processing. Eur J Immunol 1992; 22:1919–1925.
76. Shi HZ, Humbles A, Gerard C, Jin Z, Weller PF. Lymph node trafficking and antigen presentation by endobronchial eosinophils. J Clin Invest 2000; 105:945–953.
77. Dessein AJ, Parker WL, James SL, David JR. IgE antibody and resistance to infection. I. Selective suppression of the IgE antibody response in rats diminishes the resistance and the eosinophil response to *Trichinella spiralis* infection. J Exp Med 1981; 153:423–436.
78. Pritchard DI, Quinnell RJ, Walsh EA. Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. Parasite Immunol 1995; 17:71–75.
79. Dunne DW, Butterworth AE, Fulford AJ, Kariuki HC, Langley JG, Ouma JH, Capron A, Pierce RJ, Sturrock RF. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. Eur J Immunol 1992; 22:1483–1494.
80. Dunne DW, Webster M, Smith P, Langley JG, Richardson BA, Fulford AJ, Butterworth AE, Sturrock RF, Kariuki HC, Ouma JH. The isolation of a 22 kDa band after SDS-PAGE of *Schistosoma mansoni* adult worms and its use to demonstrate

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that IgE responses against the antigen(s) it contains are associated with human resistance to reinfection. Parasite Immunol 1997; 19:79–89.
81. Hagan P, Blumenthal UJ, Dunn D, Simpson AJ, Wilkins HA. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. Nature 1991; 349: 243–245.
82. Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. Eur J Immunol 1991; 21:2679–2686.
83. Satti MZ, Lind P, Vennervald BJ, Sulaiman SM, Daffalla AA, Ghalib HW. Specific immunoglobulin measurements related to exposure and resistance to *Schistosoma mansoni* infection in Sudanese canal cleaners. Clin Exp Immunol 1996; 106:45–54.
84. Viana IR, Correa-Oliveira R, Carvalho OdS, Massara CL, Colosimo E, Colley DG, Gazzinelli G. Comparison of antibody isotype responses to *Schistosoma mansoni* antigens by infected and putative resistant individuals living in an endemic area. Parasite Immunol 1995; 17:297–304.
85. Zhang Z, Wu H, Chen S, Hu L, Xie Z, Qiu Y, Su C, Cao JP, Wu Y, Zhang S, Wu G. Association between IgE antibody against soluble egg antigen and resistance to reinfection with *Schistosoma japonicum*. Trans R Soc Trop Med Hyg 1997; 91:606–608.
86. Amiri P, Haak-Frendscho M, Robbins K, McKerrow JH, Stewart T, Jardieu P. Anti-immunoglobulin E treatment decreases worm burden and egg production in *Schistosoma mansoni*-infected normal and interferon gamma knockout mice. J Exp Med 1994; 180:43–51.
87. Chang TW. The pharmacological basis of anti-IgE therapy. Nat Biotechnol 2000; 18:157–162.
88. Heusser C, Jardieu P. Therapeutic potential of anti-IgE antibodies. Curr Opin Immunol 1997; 9:805–813.
89. Milgrom H, Fick RB, Jr., Su JQ, Reimann JD, Bush RK, Watrous ML, Metzger WJ. Treatment of allergic asthma with monoclonal anti-IgE antibody. rhuMAb- E25 Study Group. N Engl J Med 1999; 341:1966–1973.
90. Bell RG. IgE, allergies and helminth parasites: a new perspective on an old conundrum. Immunol Cell Biol 1996; 74:337–345.

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6

Eosinophil IgE Receptors

Controversy and Consensus

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I. Introduction

During the past decade, considerable new information has been obtained about the function of the eosinophil and its role in human diseases. Presently, the eosinophil is recognized as a proinflammatory granulocyte implicated in protection against parasitic infection and believed to play an important role in allergic diseases, such as bronchial asthma, allergic rhinitis, and atopic dermatitis (1). The eosinophil is an important source of cytotoxic cationic proteins, such as major basic protein (MBP), eosinophil peroxidase, and eosinophil cationic protein, that have the potential of a two-edged sword: on the one hand, protecting the host against overwhelming helminth infections but, on the other hand, causing host tissue damage (2). Eosinophils can also induce inflammation by releasing lipid mediators, oxygen metabolites, and cytokines (2). Numerous studies have shown the association of eosinophils and various human parasitic and allergic diseases. For example, the most common cause of eosinophilia worldwide today is probably infection with helminths (3). Analyses of patients infected with *Onchocerca volvulus* have shown striking deposition of the eosinophil granule MBP around degenerating microfilaria (4). In allergic diseases, eosinophilic and lymphocytic infiltration in the epithelium and lamina propria of the airways have been consis-

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tent findings even in mild and stable asthma (5). Indeed, correlations have been observed between the number of infiltrating eosinophils and asthma disease severity (5). Pulmonary segmental allergen challenge in allergic individuals causes eosinophil recruitment into the airways; this is associated with the release of eosinophil granule proteins and an increase in vascular permeability (6,7). In spite of these strong associations among eosinophils, their cytotoxic granule proteins, and human diseases, the activation mechanism(s) of eosinophils in vivo has been largely unknown.

Helminth infections and allergic diseases are characteristically associated not only with peripheral blood and tissue eosinophilia, but also with high levels of both total and antigen-specific IgE antibodies. IgE antibody may be involved in diseases in three ways. First, it is well known that IgE-dependent mast cell activation and release of histamine and other inflammatory mediators, such as prostaglandins and leukotrienes, from these cells is the central element in anaphylactic and immediate hypersensitivity reactions (8). Further, upon activation through IgE receptors, human mast cells and basophils have been shown to produce cytokines, such as interleukin (IL)-4 and IL-5, which are potentially important to recruit eosinophils and to cause chronic allergic inflammation (9). Second, the interaction of IgE antibody and IgE receptors on antigen presenting cells, such as CD23 on B cells and the high-affinity IgE receptor (FcεRI) on Langerhans cells and monocytes, may present antigen to and stimulate T cells, resulting in a continuous activation of the immune system (10). Finally, IgE may mediate killing of the invading helminth and host cell damage by acting as a ligand for antibody-dependent cell-mediated cytotoxicity (ADCC) by macrophages and other immune cells (11). In fact, in immunoepidemiological studies, Hagan et al. demonstrated a significant correlation between the production of antischistosome IgE antibodies and the acquisition of immunity against reinfection to *Schistosoma haematobium* (12). In allergic diseases such as bronchial asthma, there is a close correlation between serum IgE levels and the prevalence and severity of the diseases (13). Thus, there is now converging evidence to support a role for IgE in resistance to helminthic infection and in the pathophysiology of allergic diseases in humans. Therefore, it is reasonable to speculate that IgE is involved in the activation of eosinophils in these diseases. In this chapter we will review how eosinophil IgE receptor research has progressed, describe current knowledge regarding the low-affinity IgE receptor (FcεRII/CD23) and the high-affinity IgE receptor (FcεRI) on human eosinophils, and identify potential problems and future directions.

II. History of IgE Receptors on Eosinophils

Early studies on the killing of schistosomula in vitro by human eosinophils involved the use of cells purified from normal or slightly eosinophilic individuals,

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together with heat-inactivated sera from individuals with schistosomiasis (14). The results of these studies suggested that the reaction could involve IgG and be independent of complement. Further analysis showed that IgG1 and IgG3 subclasses effectively mediate ADCC by human eosinophils, while IgM, IgG2, and IgG4 are not only inactive, but indeed block the effects of the active subclasses (15). A quite separate phenomenon was observed with eosinophils with low-density, so-called ''hypodense'' eosinophils, that are recovered from individuals with very high eosinophil counts. Receptors for IgE were identified on both rat and human eosinophils (16), and ''hypodense'' human eosinophils were shown to kill schistosomula in the presence of IgE (17). Subsequently, much work has been done on this eosinophil IgE receptor, which currently appears to be similar to the low affinity IgE receptor (FcεRII) expressed on B cells (CD23) (18–22). Eosinophils from patients with eosinophilia express another low-affinity IgE-binding molecule belonging to the S-type lectin family, called Mac-2/ε-binding protein (23). The cytotoxic function of eosinophils was abolished by the antibody against this molecule (23). More recently in 1994, Gounni et al. described FcεRI on eosinophils from patients with marked eosinophilia (24). The evidence to support this claim was comprehensive and included inhibition of [^{125}I] IgE binding to eosinophils by anti-FcεRI α-chain monoclonal antibody (mAb) (clone 15.1), surface expression of FcεRI α chain by flow cytometry, immunostaining of tissue eosinophils with 15.1, the demonstration of FcεRI α-, β-, and γ-chain transcript, the release of eosinophil granule proteins after stimulation of eosinophils with 15.1, and the inhibition of IgE-dependent eosinophil ADCC against *Schistosoma* targets with 15.1. Similar observations were found recently with rat eosinophils obtained from peritoneal cavities of animals injected with thioglycolate (25). Altogether, these findings suggested that human eosinophils express three receptors for IgE, including FcεRI, FcεRII, and Mac-2, and lead to the conclusion that IgE-mediated activation of eosinophils could be potentially important for host defense and pathophysiology of diseases.

However, this seemingly strong association between human and rat eosinophils and IgE-mediated diseases has been unclear in mice. For example, analyses of the effects of antibodies to IL-5 on helminth-induced eosinophilia have shown that anti-IL-5 suppresses blood eosinophilia and eosinophil infiltration into the tissues of parasitized animals. However, ablation of eosinophilia by anti-IL-5 was not associated with a diminution of resistance in mice infected with *S. mansoni* (26) or with *Trichinella spiralis* (27). These findings suggest that eosinophils are not involved in the expression of immunity to these parasites, at least in these mouse models. In contrast, mice infected with *Trichuris muris* showed the exactly opposite findings. In this infection, the resistance to infection was associated with the production of Th2 cytokines, such as IL-4 and IL-5, tissue eosinophilia, and intestinal IgA production (28). Furthermore, in murine asthma models, the experiments using anti-IL-5 showed that neither IL-5 nor eosinophils were required for airway hyperresponsiveness in BALB/c mice sensitized and challenged with

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ovalbumin (OVA) (29). In contrast, C57BL/6 mice rendered IL-5 deficient by homologous gene recombination failed to develop eosinophil infiltration into the lungs, airway hyperresponsiveness, and lung damage, all of which were seen in littermate controls sensitized and exposed to OVA (30). These contrasting findings among murine models of eosinophilic disorders and some inconsistencies between the findings in humans and mice have led to a recognition of potential difference(s) between human and mouse eosinophilic leukocytes.

Previously, Lopez et al. described the distribution of Fc receptors on mouse eosinophils isolated from mice infected with the cestode *Mesocestoides corti* (31,32). In these reports the authors determined that mouse eosinophils, similar to human eosinophils, expressed the type II Fc receptor for IgG (FcγRII), but they were unable to detect any IgE binding to mouse eosinophils. More recently, Jones et al. thoroughly examined eosinophils obtained by bronchoalveolar lavage (BAL) from the lungs of CBA/J mice infected with *Toxocara cani* by flow cytometry (33). They found that mouse eosinophils are negative for surface IgM (sIgM), sIgA, sIgE, and FcεRII but are positive for sIgG1 and FcγRII. Furthermore, they showed that culturing eosinophils for 24 or 48 hours with exogenous IgE or IL-4 or both did not induce IgE binding capacity or FcεRII expression. De Andres et al. expanded these studies by including FcεRI and Mac-2 and by examining mRNA transcript and receptor-mediated cellular function (34). Furthermore, de Andres et al. examined murine eosinophils from two sources, namely eosinophils isolated from liver granuloma of CBA mice infected with *S. mansoni* and bone marrow cells isolated from BALB/c mice and cultured with a combination of eosinophil growth factors. The results obtained from these two different sources are virtually identical with regard to the lack of IgE receptor expression on murine eosinophils, and they could not detect any surface expression of FcεRII or Mac-2 or binding of murine IgE to the cells. Reverse transcription (RT)-PCR analyses did not detect mRNA transcript for the α chain of FcεRI or FcεRII but did detect Mac-2 mRNA. Furthermore, in vitro culture of granuloma eosinophils did not induce IgE binding or expression of IgE receptors. In contrast to the lack of IgE receptors, functioning IgG receptors, including FcγRIIb and FcγRIII, were detected on granuloma eosinophils, consistent with previous observations by others. Therefore, murine eosinophils seem to lack IgE receptors and need to utilize the FcγR, complement receptors, and/or possibly FcαR to carry out antigen-dependent cellular functions; this may explain the discrepancies among findings made in humans and mice.

III. Low-Affinity IgE Receptor (FcεRII/CD23) on Human Eosinophils

The initial report for the expression of low-affinity IgE receptors on human eosinophils goes back to the mid-1980s. In this report (18), the investigators generated

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an IgM mAb, called BB10, by immunization of mice with human ''hypodense'' eosinophils. This BB10 mAb inhibited IgE-dependent cytotoxicity of human eosinophils and platelets. The competition experiments revealed a cross-inhibition between the binding of BB10 and IgE in eosinophils, platelets, and monocytes, suggesting the specificity of BB10 for the IgE-binding site(s) of these cell types. Subsequently, the same investigators found the inhibitory effects of both polyclonal and monoclonal antibodies, cross-reacting with B-cell FcεRII/CD23, on the IgE-mediated cytotoxic functions of eosinophils, suggesting that eosinophils likely express this receptor (20). Furthermore, the mAb 135 (an anti B-cell CD23) bound to molecules immunoprecipitated by BB10 and vice versa, both on eosinophils and on B cells, suggesting the existence of cross-reactive molecules between eosinophil FcεRII and B cell CD23. Using flow cytometry and different anti-CD23 mAbs, a low expression of CD23 was observed on human eosinophils with a large heterogeneity among the patients (22). In a recent study the presence of CD23 mRNA, totally homologous in sequence with B-cell CD23, was detected by in situ hybridization and RT-PCR (35); both the CD23a and CD23b isoforms were detected. These results led to the conclusions that human eosinophils synthesize and express the CD23 molecule; this molecule is homologous to B-cell CD23 and is involved in the IgE-mediated functions of human eosinophils.

Although the earlier observations on the presence of FcεRII/CD23 in human eosinophils seemed convincing, several controversies were raised recently. For example, Seminario et al. (36), Kita et al. (37), Saini et al. (38), and Sano et al. (39) studied blood and, in some cases, BAL eosinophils from patients with a wide variety of diseases, including asthma, allergic rhinitis, atopic dermatitis, parasitic infections, and marked eosinophilia, using several different anti-CD23 mAb and flow cytometry. These investigators were not able to detect CD23 on the surface of eosinophils, while CD23 was detected on monocytes from the same patients (37,38). On the other hand, one of these reports (39) observed that permeabilized eosinophils contain a significant intracellular pool of FcεRII that is similar to B-cell FcεRII and that it is rapidly and transiently mobilized to the cell surface upon stimulation in vitro. The physiological significance of these findings is still unknown. For example, whether CD23 is capable of more sustained surface expression under certain in vivo conditions than the expression elicited under experimental conditions needs to be elucidated. Eosinophils infiltrating into the lesions of patients with atopic dermatitis were also positively stained with anti-CD23 mAb (40); however, the authors did not discriminate whether this molecule exists within the cells or is expressed on the cell's surface. Thus, it is possible that, in certain diseases, CD23 may be expressed by human eosinophils. However, the frequency of these diseases may be rare, as judged by data indicating the lack of CD23 expression in various human diseases (36–39). It is also still unclear whether there are any circumstances in which eosinophil CD23 plays roles in eosinophil activation and disease process.

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IV. High-Affinity IgE Receptor (FcεRI) on Human Eosinophils

The demonstration that FcεRI could be detected on Langerhans cells in the skin (41) suggested that it might be expressed on other cell populations. Earlier, as described above, eosinophils were considered to express only the low-affinity IgE receptor (FcεRII) (16–20). However, studies by Gounni et al. (24) demonstrated that eosinophils from patients with marked eosinophilia express FcεRI and that they release inflammatory mediators and exert cytotoxicity to helminths through this receptor. In these studies, a mAb (clone 15.1) directed to the α chain of FcεRI (FcεRIα) was used in flow cytometry to detect surface expression of the FcεRI. FcεRI was not only detected on blood eosinophils but also on skin eosinophils from patients with atopic dermatitis by immunostaining skin biopsy specimens. Furthermore, mRNA for α, β, and γ chains of FcεRI was detected by Northern blot or RT-PCR. The mAb to FcεRI induced degranulation of eosinophils and also was able to inhibit [^{125}I] IgE binding and IgE-mediated cytotoxicity to *S. mansoni* targets. Altogether, these findings represented a comprehensive study and suggested that a functional FcεRI is expressed on human eosinophils. Subsequent studies by other investigators on patients with allergic diseases showed that human tissue eosinophils from allergen-induced late-phase skin reactions expressed mRNA for the α, β, and γ subunits of FcεRI and gave positive staining for anti-FcεRIα antibody (42,43). Eosinophils reactive with anti-FcεRIα were also found in skin and bronchial tissues from patients with atopic dermatitis (40) and asthma (44), respectively. Furthermore, increased numbers of FcεRIα mRNA- and FcεRIα protein-positive cells were detected in the nasal mucosa of patients with allergic asthma after allergen challenge (45). Thus, it has been reasonably concluded that eosinophils in patients with allergic diseases and diseases associated with eosinophilia express FcεRI and that this receptor may play a role in eosinophil activation and release of inflammatory mediators.

However, there are caveats in this conclusion. Most of the patient studies were performed by histological examinations using in situ hybridization and immunocytochemical techniques, techniques that do not allow differentiation between cell surface versus intracellular expression of the protein. Therefore, except for the first report (24), little is known about the actual expression of FcεRI protein on the surface of eosinophils. Furthermore, the functional significance of the FcεRI expression by eosinophils from patients with allergic diseases has not been elucidated. Subsequent studies addressing these key questions revealed contrasting findings from earlier reports. For example, investigators carefully and repeatedly examined peripheral blood and BAL eosinophils using specimens from various diseases associated with eosinophils, such as asthma, atopic dermatitis, parasitic infections, and the idiopathic and familial hypereosinophilic syndromes; they used several anti-FcεRI mAb (clones 22E7, 15.1, and 15A5) and several techniques, such as flow cytometry and cell surface biotinylation (36–

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39,46). The results of these extensive studies were consistent, and no or negligible expression of FcεRI was detected on the cell surface of human eosinophils. Using a highly sensitive biotinylated IgE probe, we were able to estimate the surface expression level of FcεRI on blood eosinophils in patients with allergic rhinitis; it was very small, reaching at most 0.5% of the levels expressed by basophils from the same donors (37). In addition, both other investigators (39) and we (37) failed to detect any effector functions of eosinophils, such as degranulation, superoxide production, and leukotriene C4 production, mediated by FcεRI, while basophil histamine release was observed in the same experimental conditions. Nonetheless, these eosinophils released appreciable amounts of granule proteins in response to stimuli mediated through FcγR or FcαR (37,39). Thus, while eosinophils from patients with allergic diseases and asthma may transcribe the mRNA for FcεRI subunits and make the proteins, they likely have extremely low surface expression of FcεRI. Such an eosinophil FcεRI may have no or minimal functions.

FcεRI expression is regulated by at least two factors, namely IL-4 and IgE. A direct correlation was observed between serum IgE levels and FcεRI present on human basophils (47) and mast cells (48). These observations were extended by Sihra et al. (46), who showed the correlation also exists for monocytes; however, they and other investigators (36,38) failed to show such a correlation for eosinophils. We have studied blood eosinophils from patients with a single disease, allergic rhinitis, using a sensitive biotinylated probe (37). In this group, there was a significant direct correlation between serum IgE levels and eosinophil expression of FcεRI (37). Importantly, however, the expression level of FcεRI was barely detectable by the conventional indirect immunofluorescence method even in the patient with the highest level of serum IgE. IL-4 was shown to upregulate FcεRI expression on human mast cells derived from cord blood (49) and fetal liver (50). Terada et al. (51) showed that blood eosinophils from patients with allergic rhinitis also had increased mRNA for the α chain of FcεRI after treatment with IL-4, whereas no effect was detected at the protein level. Seminario et al. (36) and Smith et al. (52) cultured eosinophils with IgE, IL-4, other cytokines/growth factors, and tissue matrix proteins for up to 11 days in an attempt to induce expression of FcεRI; however, none of the conditions tested were able to promote surface expression of FcεRI by human eosinophils. Thus, the cell surface expression of the FcεRI complex by human eosinophils is not likely to be upregulated by IgE and IL-4 as it is in mast cells and basophils.

It may be important to note that there seems to be a dissociation between the synthesis of FcεRI subunits and the cell surface expression of this receptor in human eosinophils. Indeed, Smith et al. (52) confirmed the presence of intracellular FcεRIα protein, using flow cytometry to analyze permeabilized cells as well as immunohistochemistry to directly visualize cytospin preparations, but negligible FcεRIα protein was detectable on the cell surface. Furthermore, Semi-

nario et al. (36) used immunoprecipitation and Western blot analysis to show that eosinophils have subunit proteins for FcεRI, including FcεRIα and FcεRIγ, within the cells, but that FcεRIα was not expressed on the surface. Instead, the investigators found that FcεRIα protein was spontaneously released into the supernatant of cultured eosinophils (36). Interestingly, FcεRIγ protein was expressed on the eosinophil's surface (36); this protein may be involved in the formation of other FcR on eosinophils, such as FcγRIII (CD16). The meaning and significance of released soluble FcεRIα protein, that is, whether it could act to downregulate cellular responses to IgE by binding free IgE and decreasing its serum levels, need further study. At present there is no explanation for why eosinophils release FcεRIα protein instead of expressing it on their cell surface. Previous reports have shown that truncation of the transmembrane and cytoplasmic domains of FcεRIα results in a protein secreted by Chinese hamster ovary cells (53). Therefore, future investigations need to examine the mRNA transcript and protein sequence of eosinophil FcεRIα and to determine the molecular mechanisms of FcεRIα release by human eosinophils.

V. Cautions, Potential Problems, and Future Directions

The controversies concerning eosinophil IgE receptors have taught us several important lessons. First, transcription of mRNA or even the presence of synthesized receptor protein within the cells does not necessarily indicate the expression of the receptor on cell surfaces. Potential discrepancies between receptor expression and actual receptor function need to be resolved.

Second, expression of IgE receptors on human eosinophils and IgE-dependent functions of these receptors may not be commonly seen in eosinophils. The initial studies regarding the presence of IgE receptors on human eosinophils have utilized eosinophils from patients with marked eosinophilia, including patients with hypereosinophilic syndrome and diseases associated with skin disorders and lymphomas (18,24). There are no data from these same investigators to document expression of IgE receptors on eosinophils from healthy donors (no eosinophilia) and from patients with mild to moderate eosinophilia due to more common conditions, such as allergy and asthma. Later studies by other investigators, which sought IgE receptors in these common conditions, found no or negligible surface expression of FcεRI and FcεRII by human eosinophils (36–39,46,52,54). One way to reconcile these observations of the lack of surface FcεRI in these reports with that of Gounni et al. (24) is to conclude that eosinophils from atopic individuals are substantially different from eosinophils in patients with potential hematological disorders. Because blood and tissue eosinophils, even those from atopic subjects, express mRNA for three subunits of FcεRI (42,43), there may be post-translational mechanisms present in rare hematological disorders that are not ob-

served with eosinophils in allergic diseases. Nevertheless, recent observations in atopic subjects (37,52) suggest that FcεRI is unlikely to be important in eosinophil activation and degranulation in allergic diseases.

Third, in association with the issues described above, expression of IgE receptors on eosinophils may be tightly regulated by various environmental factors. For example, in humans the expression of FcεRII was limited to eosinophils from patients with marked eosinophilia and, even in this group, to a unique cell population of ''hypodense'' eosinophils (55). Furthermore, expression of mRNA transcript and receptor protein for FcεRI and FcεRII is likely regulated by IL-4 and IgE (47–50,56). Therefore, diseases or tissue environments or both with abundant IL-4 and IgE, as well as local milieu enriched in cytokines, growth factors, and co-stimulatory molecules, may favor the expression of IgE receptors on eosinophils. In fact, by using mice selected for heightened production of IgE, Eum et al. concluded that both the recruitment of eosinophils to the airways and high IgE titers are required for lung pathology of allergic BP2 mice (57), and they speculated on a possible IgE-driven activation of eosinophils in this mouse model. In addition, in contrast to mice, the expression of IgE receptors on rat eosinophils is more widely accepted (16,25,58). Thus, IgE receptor expression may be disease-, tissue-, species-, and/or strain-specific, and the observations obtained with certain conditions should not be generalized. Therefore, we cannot exclude the possibility that tissue eosinophils, as opposed to blood eosinophils, may express functional cell surface FcεRI in humans. Nonetheless, the lack of this molecule in eosinophils cultured in a variety of conditions, including enrichment with IL-4 and IgE (36,52), and in eosinophils from BAL fluids after allergen challenge (36), raises the large question as to other optimal conditions able to induce surface expression of FcεRI by human eosinophils.

Finally, several published articles from investigators who studied human eosinophil IgE receptors need to be reconciled. In 1988, the eosinophil IgE receptor was originally found to have a low affinity (Kd of 10^{-7} M) and to possess a molecular weight that corresponded to that of FcεRII (19). IgE binding and IgE-dependent ADCC to schistosomula were completely inhibited by antibody to either FcεRII (18) or Mac-2 (23). Hence, earlier studies demonstrated evidence for expression of the low-affinity IgE receptor without any evidence of the high-affinity IgE receptor, FcεRI. However, later in 1994 the same investigators reported the presence of the FcεRI on human eosinophils, and all the IgE-dependent functions of human eosinophils were explained by this receptor (24). Therefore, the reports on eosinophil expression of FcεRI, FcεRII, and Mac-2 need to be reconciled on the relative expression and function of each receptor.

In summary, as shown in Table 1, human eosinophils from patients with allergy and asthma transcribe mRNA and contain considerable amounts of intracellular but no or negligible surface FcεRIα. Eosinophils do not seem to express FcεRII (CD23) surface protein either. Unlike mast cells and basophils, investiga-

Table 1 Detection of Expression of FcεR by Eosinophils

Principal author (Ref.)	Year	Species	Source	Disease/Treatment	mRNA[a]	Protein	Receptor
FcεRI							
Gounni (24)	1994	Human	Blood	Eosinophilia	α(+), β(+), γ(+)	α(+)	(+)
Terada (51)	1995	Human	Blood, tissue	Allergic rhinitis	α(+)	α(+/−)	(−)
Tanaka (40)	1995	Human	Tissue	Atopic dermatitis		α(+)	
Humbert (44)	1996	Human	Tissue	Asthma		α(+)	
Barata (42)	1997	Human	Tissue	Allergy	α(+), β(+), γ(+)	α(+)	
Ying (43)	1998	Human	Tissue	Allergy	α(+), β(+), γ(+)	α(+)	
Sihra (46)	1997	Human	Blood	Allergy			(−, +/−)
Rajakulasingam (45)	1998	Human	BAL	Asthma	α(+)	α(+)	
Seminario (36)	1999	Human	Blood, BAL	Allergy, eosinophilia		α(+), β(−), γ(+)	(−)
Kita (37)	1999	Human	Blood	Allergic rhinitis			(−, +/−)
Saini (38)	2000	Human	Blood	Allergy, eosinophilia			(−, +/−)
Smith (52)	2000	Human	Blood	Allergy	α(+), β(+), γ(+)	α(+)	(−, +/−)
de Andres (34)	1997	Mouse	Tissue, BM	Schistosomiasis, normal	α(−)		
Dombrowicz (25)	2000	Rat	Peritoneal	Thioglycolate injection		α(+), β(−), γ(+)	(+)
FcεRII[b]							
Capron (18)	1986	Human	Blood, BAL	Eosinophilia		(+)	(+)
Hartnell (54)	1989	Human	Blood	Allergy			(−)
Tanaka (40)	1995	Human	Tissue	Atopic dermatitis		(+)	
Gounni (35)	1998	Human	Blood	Eosinophilia	(+)	(+)	
Sano (39)	1999	Human	Blood	Allergy		(+)	(+/−)
Seminario (36)	1999	Human	Blood, BAL	Allergy, eosinophilia			(−)
Kita (37)	1999	Human	Blood	Allergic rhinitis			(−)
Saini (38)	2000	Human	Blood	Allergy, eosinophilia			(−)
Jones (33)	1994	Mouse	BAL	*Toxocara* infection			(−)
de Andres (34)	1997	Mouse	Tissue, BM	Schistosomiasis, normal	(−)		(−)
Mac-2							
Truong (23)	1993	Human	Blood	Eosinophilia		(+)	(+)
de Andres (34)	1997	Mouse	Tissue, BM	Schistosomiasis, normal	(+)		(−)

[a] α, β, and γ denote α, β, and γ chain of FcεRI, respectively. (+/−) denotes minimal or indefinite expression.

[b] The citations of FcεRII are a partial listing.

BAL, Bronchoalveolar lavage; BM, bone marrow.

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tors were not able to demonstrate upregulation of cell surface FcεRI or FcεRII using IgE and IL-4. Therefore, it may be reasonable to conclude that the functional significance of eosinophil IgE receptors in human allergic diseases and eosinophilic disorders is highly questionable. Interestingly, the FcεRI α chain is released from eosinophils into the supernatant during culture. Whether the eosinophil transiently expresses FcεRI on its surface, which is then cleaved, whether the FcεRI α chain is directly secreted, and whether this soluble FcεRI α chain bears any biological significance still need to be resolved. Further studies of IgE receptors on mouse and human eosinophils may solve the existing controversies and help to interpret and understand the pathophysiological mechanisms of human eosinophilic disorders, the roles of eosinophils in human immunity, and their murine models.

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References

1. Butterfield JH, Leiferman KM, Gleich GJ. Eosinophil-associated diseases. In: Frank MM, Austen KF, Claman HN, Unanue ER, eds. Samter's Immunologic Diseases. Boston: Little, Brown and Co., 1994:501–527.
2. Kita H, Adolphson CR, Gleich GJ. Biology of eosinophils. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, eds. Allergy: Principles and Practice, 5th ed. St. Louis: Mosby-Year Book, Inc., 1998:242–260.
3. Weller PF. Eosinophilia in travelers. Med Clin North Am 1992; 76:1413–1432.
4. Kephart GM, Gleich GJ, Connor DH, Gibson DW, Ackerman SJ. Deposition of eosinophil granule major basic protein onto microfilariae of *Onchocerca volvulus* in the skin of patients treated with diethylcarbamazine. Lab Invest 1984; 50:51–61.
5. Bousquet JB, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J, Godard P, Michel FB. Eosinophilic inflammation in asthma. N Engl J Med 1990; 323:1033–1039.
6. Sedgwick JB, Calhoun WJ, Gleich GJ, Kita H, Abrams JS, Schwartz LB, Volovitz B, Ben-Yaakov M, Busse WW. Immediate and late allergic airway response to segmental antigen. Characterization of eosinophil and mast cell mediators. Am Rev Respir Dis 1991; 144:1274–1281.
7. Collins DS, Dupuis R, Gleich GJ, Bartemes KR, Ko YY, Pollice M, Albertine KH, Fish JE, Peters SP. Immunoglobulin E-mediated increase in vascular permeability correlates with eosinophilic inflammation. Am Rev Respir Dis 1993; 147:677–683.

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8. Galli SJ, Gordon JR, Wershil BK. Cytokine production by mast cells and basophils. Curr Opinion Immunol 1991; 3:865–872.
9. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, Heusser CH, Howarth PH, Holgate ST. Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. Am J Respir Cell Mol Biol 1994; 10:471–480.
10. Mudde GC, Bheekha R, Bruijnzeel-Koomen CA. IgE-mediated antigen presentation. Allergy 1995; 50:193–199.
11. Capron A, Dessaint JP, Haque A, Capron M. Antibody-dependent cell-mediated cytotoxicity against parasites. Progr Allergy 1882; 31:234–267.
12. Hagan P, Blumenthal UJ, Dunn D, Simpson AJ, Wilkins HA. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. Nature 1991; 349: 243–245.
13. Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. N Engl J Med 1991; 325:1067–1071.
14. Butterworth AE, Sturrock RF, Houba V, Mahmoud AA, Sher A, Rees PH. Eosinophils as mediators of antibody-dependent damage to schistosomula. Nature 1975; 256:727–729.
15. Khalife J, Dunne DW, Richardson BA, Mazza G, Thorne KJ, Capron A, Butterworth AE. Functional role of human IgG subclasses in eosinophil-mediated killing of schistosomula of *Schistosoma mansoni*. J Immunol 1989; 142:4422–4427.
16. Capron M, Capron A, Dessaint JP, Torpier G, Johansson SG, Prin L. Fc receptors for IgE on human and rat eosinophils. J Immunol 1981; 126:2087–2092.
17. Capron M, Spiegelberg HL, Prin L, Bennich H, Butterworth AE, Pierce RJ, Ouaissi MA, Capron A. Role of IgE receptors in effector function of human eosinophils. J Immunol 1984; 132:462–468.
18. Capron M, Jouault T, Prin L, Joseph M, Ameisen JC, Butterworth AE, Papin JP, Kusnierz JP, Capron A. Functional study of a monoclonal antibody to IgE Fc receptor (Fc epsilon R2) of eosinophils, platelets, and macrophages. J Exp Med 1986; 164:72–89.
19. Jouault T, Capron M, Balloul JM, Ameisen JC, Capron A. Quantitative and qualitative analysis of the Fc receptor for IgE (FcεRII) on human eosinophils. Eur J Immunol 1988; 18:237–241.
20. Grangette C, Gruart V, Ouaissi MA, Rizvi F, Delespesse G, Capron A, Capron M. IgE receptor on human eosinophils (FcεRII). Comparison with B cell CD23 and association with an adhesion molecule. J Immunol 1989; 143:3580–3588.
21. Yokota A, Kikutani H, Tanaka T, Sato R, Barsumian EL, Suemura M, Kishimoto T. Two species of human Fc epsilon receptor II (FεRII/CD23): tissue-specific and IL-4-specific regulation of gene expression. Cell 1988; 55:611–618.
22. Capron M, Truong MJ, Aldebert D, Gruart V, Suemura M, Delespesse G, Tourvieille B, Capron A. Heterogeneous expression of CD23 epitopes by eosinophils from patients. Relationships with IgE-mediated functions. Eur J Immunol 1991; 21:2423–2429.
23. Truong MJ, Gruart V, Liu FT, Prin L, Capron A, Capron M. IgE-binding molecules (Mac-2/ε BP) expressed by human eosinophils. Implication in IgE-dependent eosinophil cytotoxicity. Eur J Immunol 1993; 23:3230–3235.

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24. Gounni AS, Lamkhioued B, Ochiai K, Tanaka Y, Delaporte E, Capron A, Kinet JP, Capron M. High-affinity IgE receptor on eosinophils is involved in defence against parasites. Nature 1994; 367:183–186.
25. Dombrowicz D, Quatannens B, Papin JP, Capron A, Capron M: Expression of a functional FcεRI on rat eosinophils and macrophages. J Immunol 2000; 165:1266–1271.
26. Sher A, Coffman RL, Hieny S, Scott P, Cheever AW. Interleukin 5 is required for the blood and tissue eosinophilia but not granuloma formation induced by infection with *Schistosoma mansoni*. Proc Natl Acad Sci USA 1990; 87:61–65.
27. Herndon FJ, Kayes SG. Depletion of eosinophils by anti-IL-5 monoclonal antibody treatment of mice infected with *Trichinella spiralis* does not alter parasite burden or immunologic resistance to reinfection. J Immunol 1992; 149:3642–3647.
28. Else KJ, Hultner L, Grencis RK: Cellular immune responses to the murine nematode parasite *Trichuris muris*. II. Differential induction of TH-cell subsets in resistant versus susceptible mice. Immunology 1992; 75:232–237.
29. Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener-Kronish JP, Locksley RM. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity [see comments]. J Exp Med 1996; 183:109–117.
30. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model [see comments]. J Exp Med 1996; 183:195–201.
31. Lopez AF, Strath M, Sanderson CJ. Mouse immunoglobulin isotypes mediating cytotoxicity of target cells by eosinophils and neutrophils. Immunology 1983; 48:503–509.
32. Lopez AF, Battye FL, Vadas MA. Fc receptors on mouse neutrophils and eosinophils: antigenic characteristics, isotype specificity and relative cell membrane density measured by flow cytometry. Immunology 1985; 55:125–133.
33. Jones RE, Finkelman FD, Hester RB, Kayes SG. *Toxocara canis*: failure to find IgE receptors (FcεR) on eosinophils from infected mice suggests that murine eosinophils do not kill helminth larvae by an IgE-dependent mechanism. Exp Parasitol 1994; 78:64–75.
34. de Andres B, Rakasz E, Hagan M, McCormick ML, Mueller AL, Elliot D, Metwali A, Sandor M, Britigan BE, Weinstock JV, Lynch RG. Lack of Fc-ε receptors on murine eosinophils: implication for the functional significance of elevated IgE and eosinophils in parasitic infections. Blood 1997; 89:3826–3836.
35. Gounni SA, Lamkhioued B, Morita M, Delphine A, Marika S, Capron A, Capron M. Molecular characterization of the low-affinity IgE receptor FcεRII/CD23 expressed by human eosinophils. Int Immunol 1998; 10:395–404.
36. Seminario MC, Saini SS, MacGlashan DW Jr, Bochner BS. Intracellular expression and release of FcεRIα by human eosinophils. J Immunol 1999; 162:6893–6900.
37. Kita H, Kaneko M, Bartemes KR, Weiler DA, Schimming AW, Reed CE, Gleich GJ. Does IgE bind to and activate eosinophils from patients with allergy? J Immunol 1999; 162:6901–6911.
38. Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, MacGlashan DW Jr. The relationship between serum IgE and surface levels of FcεR on human leukocytes in various diseases: Correlation of expression with FcεRI on basophils but not on monocytes or eosinophils. J Allergy Clin Immunol 2000; 106:514–520.

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39. Sano H, Munoz NM, Sano A, Zhu X, Herrnreiter A, Choi J, Leff AR. Upregulated surface expression of intracellularly sequestered Igε receptors (FcεRII/CD23) following activation in human peripheral blood eosinophils. Proc Assoc Am Phys 1999; 111:82–91.
40. Tanaka Y, Takenaka M, Matsunaga Y, Okada S, Anan S, Yoshida H, Ra C. High affinity IgE receptor (FcεRI) expression on eosinophils infiltrating the lesions and mite patch tested sites in atopic dermatitis. Arch Dermatol Res 1995; 287:712–717.
41. Wang B, Rieger A, Kilgus O, Ochiai K, Maurer D, Fodinger D, Kinet JP, Stingl G. Epidermal Langerhans' cells from normal human skin bind monomeric IgE via FcεRI. J Exp Med 1992; 175:1353–1365.
42. Barata LT, Ying S, Grant JA, Humbert M, Barkans J, Meng Q, Durham SR, Kay AB. Allergen-induced recruitment of Fc epsilon RI+ eosinophils in human atopic skin. Eur J Immunol 1997; 27:1236–1241.
43. Ying S, Barata LT, Meng Q, Grant JA, Barkans J, Durham SR, Kay AB. High-affinity immunoglobulin E receptor (FcεRI)-bearing eosinophils, mast cells, macrophages and Langerhans' cells in allergen-induced late-phase cutaneous reactions in atopic subjects. Immunology 1998; 93:281–288.
44. Humbert M, Grant JA, Taborda-Barata L, Durham SR, Pfister R, Menz G, Barkans J, Ying S, Kay AB. High-affinity IgE receptor (FcεRI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma. Am J Respir Crit Care Med 1996; 153: 1931–1937.
45. Rajakulasingam K, Till S, Ying S, Humbert M, Barkans J, Sullivan M, Meng Q, Corrigan CJ, Bungre J, Grant JA, Kay AB, Durham SR. Increased expression of high affinity IgE (FcεRI) receptor-α chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma. Am J Respir Crit Care Med 1998; 158: 233–240.
46. Sihra BS, Kon OM, Grant JA, Kay AB. Expression of high-affinity IgE receptors (FcεRI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. J Allergy Clin Immunol 1997; 99:699–706.
47. MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, Sterbinsky SA, Hamilton RG, Lichtenstein LM. Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol 1997; 158:1438–1445.
48. Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I, Kinet JP, Galli SJ. IgE enhances mouse mast cell FcεRI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. J Exp Med 1997; 185:663–672.
49. Yamaguchi M, Sayama K, Yano K, Lantz CS, Noben-Trauth N, Ra C, Costa JJ, Galli SJ. IgE enhances Fc epsilon receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: synergistic effect of IL-4 and IgE on human mast cell Fc epsilon receptor I expression and mediator release. J Immunol 1999; 162:5455–5465.
50. Xia HZ, Du Z, Craig S, Klisch G, Noben-Trauth N, Kochan JP, Huff TH, Irani AM, Schwartz LB. Effect of recombinant human IL-4 on tryptase, chymase, and Fc epsilon receptor type I expression in recombinant human stem cell factor-dependent fetal liver-derived human mast cells. J Immunol 1997; 159:2911–2921.

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51. Terada N, Konno A, Terada Y, Fukuda S, Yamashita T, Abe T, Shimada H, Ishida K, Yoshimura K, Tanaka Y, et al. IL-4 upregulates FcεRI α-chain messenger RNA in eosinophils. J Allergy Clin Immunol 1995; 96:1161–1169.
52. Smith SJ, Ying S, Meng Q, Sullivan MHF, Barkans J, Kon OM, Sihra B, Larche M, Levi-Shaffer F, Kay AB. Blood eosinophils from atopic donors express messenger RNA for the α, β, and γ subunits of the high-affinity IgE receptor (FcεRI) and intracellular, but not cell surface, α subunit protein. J Allergy Clin Immunol 2000; 105:309–317.
53. Blank U, Ra CS, Kinet JP. Characterization of truncated alpha chain products from human, rat, and mouse high affinity receptor for immunoglobulin E. J Biol Chem 1991; 266:2639–2646.
54. Hartnell A, Walsh GM, Moqbel R, Kay AB. Phenotypic marker on human eosinophils (abstr). J Allergy Clin Immunol 1989; 83:192.
55. Capron M, Grangette C, Torpier G, Capron A. The second receptor for IgE in eosinophil effector function. Chem Immunol 1989; 47:128–178.
56. Toru H, Ra C, Nonoyama S, Suzuki K, Yata J, Nakahata T. Induction of the high-affinity IgE receptor (FcεRI) on human mast cells by IL-4. Int Immunol 1996; 8: 1367–1373.
57. Eum SY, Haile S, Lefort J, Huerre M, Vargaftig BB: Eosinophil recruitment into the respiratory epithelium following antigenic challenge in hyper-IgE mice is accompanied by interleukin 5-dependent bronchial hyperresponsiveness. Proc Natl Acad Sci USA 1995; 92:12290–12294.
58. Capron M, Bazin H, Joseph M, Capron A. Evidence for IgE-dependent cytotoxicity by rat eosinophils. J Immunol 1981; 126:1764–1768.

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7

Murine Models of Asthma

Caveats and Conclusions on the Contribution of IgE and Mast Cells to Allergic Inflammation, Airway Hyperresponsiveness, and Airflow Obstruction

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Asthma is a chronic inflammatory disease characterized by airflow obstruction, airway hyperresponsiveness, and, importantly, the infiltration of lymphocytes, eosinophils, and mast cells, which are believed to be responsible for the physiological manifestations and accompanying airway remodeling. Over a decade of research and clinical efforts have implicated numerous mediators and mechanisms to play a role in the pathogenesis of asthma. The results of studies in animals, particularly in mice, suggest that redundant mechanisms may lead to a similar outcome—asthmatic disease. Delineating the mediators of allergic asthma in animal models is important but is useful only if these studies have relevance to the pathogenesis of human asthma. To delineate such relevance of murine studies, it is important to understand the inherent differences between experimentally induced allergic pulmonary disease in mice and clinical disease in human asthmatics and appreciate the different pathogenic mechanisms that can occur between mice and humans.

To better understand the pathogenesis of human asthma, a variety of experimental approaches using murine models of allergic pulmonary inflammation have been developed. In murine models of human asthma, it is apparent that initiation of the allergic immune response is T-lymphocyte dependent (1,2). However, the predominant direct mediator(s) of allergic inflammation identified in these murine

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models appears to be variable, with numerous conflicting studies. Pulmonary eosinophilic inflammation and increased airway reactivity in these models can be suppressed by inhibiting a variety of mechanisms including elimination or suppression of T lymphocytes (1–4), elimination of T-lymphocyte helper type-2 cytokines with neutralizing antibodies or gene knockout (5), inhibition of various chemokines (6–8), inhibition of IgE (9,10), and inhibition of various adhesion molecules (11–15). Though in general these studies have been instrumental, if not critical, in delineating the function of important cytokines, chemokines, etc., it is not clear which model or data most clearly defines or best reflects the predominant pathological mechanism in human asthma, if a dominant mechanism exists. The heterogeneity of conclusions obtained from these murine studies may reflect differences in the model, strain of mouse, or the allergen and sensitization protocol utilized or simply that there are numerous, perhaps redundant, mechanisms by which allergic asthma can develop in mice (16–18). It is possible that similar redundant mechanisms exist in humans. Confidence in the relevance of these murine models may improve as efficacy data attained in mice are correlated with those attained clinically in humans using a parallel therapeutic approach targeting the same mechanism(s).

In the vast majority of human asthmatics, there is strong correlation between disease and IgE (19). The role of IgE in contributing to mast cell degranulation and subsequent pulmonary inflammation has been inferred from numerous epidemiological studies that demonstrate a relationship between levels of IgE and asthma (20–22). Disease severity correlates with serum and BAL (bronchoalveolar lavage) fluid IgE (22). The mechanistic link between the binding of serum IgE to the high affinity receptor FcεRI on mast cells that results in degranulation and anaphylaxis is also well established (23).

The defined link between human allergic asthma and IgE implies a critical role for the interaction of IgE and its Fc receptors (FcR) on mast cells, basophils, and possibly eosinophils. It is well established in humans that IgE can bind to FcεRI (CD32), the high-affinity IgE receptor, FcεRIIA (CD23), the activating ITAM motif–carrying low-affinity IgE receptor, and FcεRIIB, the inhibitory ITIM motif–carrying low-affinity IgE receptor. In humans, both mast cells and basophils express FcεRI where it exists as a tetramer of an α, β, and two γ subunits. Recent data have additionally identified the expression of FcεRI as a trimer of one α and two γ subunits on monocytes, dendritic cells, eosinophils, and platelets in atopic or hypereosinophilic human patients (24). In humans, IgE is the only antibody capable of causing mast cell degranulation.

However, in the mouse species differences in reaginic immunoglobulins and their counter-receptor type and distribution have significantly clouded the extrapolation of studies carried out in the mouse concerning the role of IgE in degranulation of mast cells. In mice and rats, mast cells and basophils express FcεRI as the tetrameric complex and also express the following Fc gamma chain

receptors: FcγRIIb1, FcγRIIb2, and FcγRIII. Thus, murine mast cells and basophils can bind to and be activated by both IgE and IgG (25,26). This significant and important difference may enable rodent mast cells to have an active role in allergic asthma models in the absence of IgE whereby IgG-allergen complexes can trigger the release of inflammatory mediators from these cells. Oshiba et al. (27) have clearly shown this by demonstrating that passive transfer of ovalbumin (OVA)-specific IgG1 can induce allergic/asthmatic pulmonary disease upon subsequent aerosol exposure to ovalbumin. It is thus critical to consider both the role of allergen-specific IgE and IgG in mice in order to accurately interpret data and accurately assess the correlation between IgE and experimentally induced allergic asthma in mice. It is important to consider this difference when trying to use data derived from murine models to understand the pathogenic mechanisms of human asthma. This perhaps has greatest significance in murine models that utilize systemic immunization with adjuvants to induce an allergic immune response as such adjuvants in mice markedly enhance T-helper type 2 responses in which high levels of both IgG1 and IgE allergen-specific antibodies are produced. Though both IgG1 and IgE are induced in these models and have a role in murine mast cell degranulation, in many published studies often only IgE is discussed or, perhaps incorrectly, deemed relevant. Since both IgG1 and IgE can induce murine mast cell degranulation, making conclusions about the dependence or independence of allergic inflammation on IgE, and therefore mast cells, in mice without considering the role of IgG1 may be inaccurate. Furthermore, because both mast cells and basophils express FcεRI, it is important to consider both cell types when studying IgE-induced allergy or anaphylaxis.

Eosinophil and basophil FcR expression is similar in humans and mice. Eosinophils from both species express FcεRII, FcγRII (A/B in humans, b1 and b2 in mice), and FcγRIII (25). Human eosinophils additionally have inducible expression of FcγRI, the high-affinity receptor for IgG (25), and express a trimeric form of FcεI in humans with hypereosinophilia (24).

With these differences in mind, we review some of the important findings identified using mouse models of human asthma with specific emphasis on the role of mast cells and IgE. We present published studies and our own data that investigate the role of mast cells and IgE in murine models of allergic inflammation. Finally, we attempt to interpret and reconcile the disparate murine data and put them in context with recent human trials using an anti-IgE antibody.

I. Evidence Indicating a Lack of Involvement of Mast Cells in Murine Asthma Models

Though the mechanism and effects of mast cell activation via cross-linked IgE is well delineated, it is apparent from numerous studies utilizing mast cell–

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deficient mice that the induction of an asthma-like phenotype in mice does not obligatorily require the presence of mast cells. For example, the anaphylactic responses previously associated with IgE-mediated mast cell activation were reported to occur in mice devoid of mast cells. Takeishi et al. (28) were the first to show that systemic antigen challenge in allergen-sensitized mast cell–deficient mice (W/Wv) or congenic normal mice resulted in identical anaphylactic responses. However, it is important to remember that the study of the mechanism of systemic anaphylaxis is not the same as the study of the induction of pulmonary allergic airway inflammation. Brusselle et al. (29) extended these findings in an active sensitization murine asthma model. In this model, W/Wv mice were not protected from developing airway inflammation after systemic OVA sensitization followed by inhalation of OVA. The W/Wv mice developed comparable BAL eosinophilia as well as total and OVA-specific IgE compared to their congenic controls. Similarly, Takeda et al. (30) reported allergic inflammation in W/Wv sensitized mice, and in addition they showed there was no difference in airway hyperresponsiveness between W/Wv and wild-type mice. These studies demonstrated that an allergic asthma-like response could be induced in mice deficient in mast cells. It should be noted that mast cell–deficient mice do have basophils and these cells may have a role in the induction of allergic pulmonary inflammation in mice. However, a defined role for basophils has not been established in these in vivo models.

II. Evidence Indicating a Lack of Involvement of IgE in Murine Asthma Models

The first study to report IgE-independent anaphylaxis occurred when the Cε-deficient mouse was generated (31). These mutant mice produce no IgE but make normal levels of other immunoglobulin isotypes. Systemic sensitization with OVA and adjuvant followed by systemic challenge resulted in anaphylaxis as measured by tachycardia, decreased pulmonary conductance and dynamic compliance, and death. The authors noted that the drop in pulmonary conductance was not as pronounced in the IgE-deficient mice as it was in the wild-type mice, suggesting that IgE may play a role in immediate airflow obstruction after antigen challenge. More direct evaluation of the role of IgE in allergic inflammation was assessed by Melhop et al. (32). Using the same Cε-deficient mice, they demonstrated that after intranasal sensitization and challenge with *Aspergillus* antigen, only the wild-type control mice developed an increase in serum IgE. Yet, both wild-type and IgE-deficient mice had equal and significant increases in BAL and airway tissue eosinophilia compared to nonsensitized controls. Furthermore, both IgE-deficient and wild-type strains of sensitized mice were hyperresponsive to methacholine challenge. Similar results were obtained by Hammelman et al. (33),

who reported that treatment with an nonanaphylactic anti-IgE antibody 2 hours prior to aerosol challenge resulted in a >80% reduction in serum IgE but an allergic inflammatory and airway reactivity response indistinguishable from the sensitized IgG control antibody-treated mice. As we have previously stated, a caveat to the interpretation of these studies lies in the fact that murine IgG is capable of binding to and activating murine mast cells.

A different approach to understanding the role of IgE was examined by Miyajima et al. (34), who blocked the high-affinity IgE receptor. The mouse was created by targeted disruption of the α subunit of the FcεRI receptor, thus eliminating confounding interpretations by avoiding disruption of the common γ subunit found in other low-affinity Fc receptors. Both control and FcεR1-deficient mice systemically sensitized and challenged with intravenous OVA exhibited anaphylaxis as described above. The authors concluded that the FcεRI receptor was not essential for the production of anaphylaxis by active sensitization in the mouse where both IgE and IgG1 are present.

A more definitive experimental model to evaluate the combined role of IgE and IgG1 in allergic inflammation has recently become available. Mice deficient in B cells produce no immunoglobulins (35). Inhalation sensitization with OVA in wild-type and B-cell–deficient mice resulted in a mild but detectable allergic inflammation in lung cell digests from both strains (36). One difference was noted—only the wild-type mice demonstrated airway responsiveness as assessed by in vitro stimulation of tracheal smooth muscle by electrical field stimulation. Thus, without the presence of either IgE or IgG1, allergic inflammation could result from antigen sensitization and challenge.

In a similar approach, using a different strain of mice and a systemic OVA sensitization followed by multiple aerosol challenges, Korsgren et al. (37) demonstrated that B-cell–deficient mice as well as wild-type sensitized controls had eosinophilic allergic inflammation and an increase in mucus-containing cells as detected by light and transmission electron microscopy. MacLean et al. (38) extended these findings by showing that, in addition to equivalent allergic inflammation as measured by cell differential, Th2-type cytokine production, and chemokine expression, B-cell–deficient mice also had equivalent airway responsiveness. The authors concluded that allergic inflammation could be induced in mice without the involvement of B cells or immunoglobulins. However, the discrepancy regarding the role of immunoglobulins in airway responsiveness between the Hamelmann et al. (36) and MacLean et al. (38) studies remains unresolved.

Further evidence that immunoglobulins are not required to produce allergic inflammation and airway hyperresponsiveness in mice has come from studies in which CD4+ Th2 cells were produced in culture from OVA-TCR transgenic donor mice. Intravenous transfer of these cultured cells alone did not produce lung pathology. However, with repeated OVA aerosol challenge, these cells were

recruited to the lung and produced allergic inflammation (39) and airway hyperresponsiveness (40) similar to actively sensitized, antigen-challenged mice. Ovalbumin specific immunoglobulins were not detected using this model (39).

III. Study I: Role of IgE and Mast Cells in a Systemic OVA Sensitization Model

The studies discussed above suggest that in mice, active sensitization can induce anaphylaxis, allergic inflammation, and airway hyperresponsiveness in the absence of mast cells or IgE. Our own research (41) attempted to verify and extend these findings by depleting IgE in mast cell–deficient mice (W/Wv). By this combined approach we hoped to ablate the direct effects of IgE on mast cells, diminish the effects of IgE on basophils and eosinophils via the low-affinity receptor CD23, and eliminate the T-cell–stimulated mast cell production of cytokines. Mice were pretreated with anti-IgE prior to active sensitization (systemic OVA plus adjuvant) and aerosol challenge, after which allergic inflammation, airway reactivity, and airflow obstruction were assessed. We observed that both W/Wv and congenic wild-type control mice developed equivalent levels of total and OVA-specific IgE in the serum (Table 1). Pretreatment with an anti-IgE antibody reduced serum total IgE to nearly undetectable levels and OVA-specific IgE to below the limit of detection of our assay. Similar results were observed in the BAL. Further evidence of the effectiveness of the anti-IgE pretreatment is suggested by the lack of IgE on B220+ BAL cells (B lymphocytes) and that aerosol exposure to the crosslinking anti-IgE antibody did not result in bronchoconstriction in sensitized mice.

Using this sensitization and challenge protocol, elimination of IgE in mast cell–deficient mice did not have a significant impact on allergic inflammation as evidenced by cellular infiltrate in BAL (Fig. 1) and lung tissue (data not shown). The BAL was characterized primarily by an increase in eosinophils and lymphocytes, with a similar cellular profile appearing in the perivascular and peribronchiolar cuffs surrounding airways in lung tissue sections. These observations were supported by a lack of difference in measurements of BAL supernatant IL-5 and eosinophil peroxidase in any of the groups of sensitized mice (Table 1). Furthermore, airflow obstruction in response to antigen challenge was observed in all but two of the mast cell–deficient and all of the wild-type sensitized mice.

As would be expected from the data above, both wild-type and W/Wv sensitized mice became hyperresponsive to methacholine compared to their respective nonsensitized controls (Fig. 2). However, nonsensitized W/Wv mice were considerably less sensitive to methacholine than were nonsensitized wild-type mice. This reduction in responsiveness of the W/Wv mice in the naïve state resulted in a methacholine response from the sensitized W/Wv mice that was

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Table 1 Serum and Lung Lavage Parameters by Group

	Nonsensitized groups				Sensitized groups			
	Wild-type controls		W/Wv mast cell–deficient		Wild-type controls		W/Wv mast cell–deficient	
Number	10	5	10	5	8	8	8	8
Anti-IgE treatment	−	+	−	+	−	+	−	+
Serum total IgE (ng/mL)	925 ± 148	—	1838 ± 305	—	3299 ± 652	275 ± 42[a]	3669 ± 565	251 ± 44[a]
Serum OVA-IgE (units)	—	—	—	—	271 ± 62	LTS	177 ± 28.6[b]	LTS
BAL total IgE (ng/mL)	9.6 ± 3.0	LTS	5.1 ± 0.7	LTS	33.9 ± 5.8	9.2 ± 3.3[a]	39.8 ± 8.0	8.6 ± 3.1[a]
Eosinophil peroxidase	0.06 ± 0.02	LTS	0.02 ± 0.02	LTS	0.32 ± 0.03[c]	0.46 ± 0.07[c]	0.26 ± 0.03[c]	0.62 ± 0.11[c]
IL-5	67.8 ± 5.4	64.0 ± 6.0	76.0 ± 5.6	78.0 ± 8.0	179.4 ± 5.4[c]	204.2 ± 13.4[c]	207.5 ± 9.2[c]	187.5 ± 24.6[c]
IL-4	27.5 ± 3.3	28.0 ± 3.8	35.0 ± 3.4	38.0 ± 3.8	31.3 ± 1.2	33.3 ± 1.4	33.3 ± 2.2	30.8 ± 4.8
Histamine	—	—	—	—	86.1 ± 12.8	6.6 ± 2.5[d]	LTS	LTS

LTS, Less than standard; —, assay not performed on this group.
[a]Different than nonsensitized mice $p < 0.001$.
[b]From wild-type control $p < 0.03$.
[c]From nonsensitized (NS) but not different from each other $p < 0.001$.
[d]From sensitized (SN) wild-type isotype control antibody-treated $p < 0.01$.

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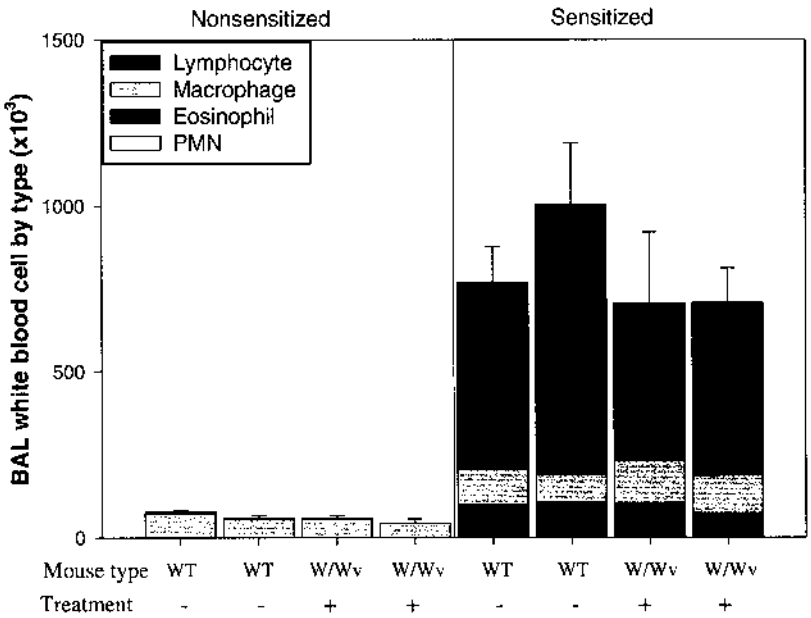

Figure 1 Neither anti-IgE nor mast cell deficiency alters BAL cellular composition. N as per Table 1. Mast cell–deficient (W/Wv) mice and wild-type control mice (WT) were sham injected or sensitized with 10 μg ovalbumin (OVA) IP and 0.75 mg of ALUM on day 0. Starting on day 14 through day 21, all mice received a 30-minute inhalation challenge of aerosolized 1% OVA delivered by a DeVilbiss ultrasonic nebulizer. Antibody-pretreated mice were injected (200 μg sc) with anti-IgE or an isotype control antibody on days −21, −14, −7, 0, 7, 14, and 18. On days 14 through 21, mice also received daily inhalations of anti-IgE or control antibody (12 mg aerosolized to completion, deposited lung dose ~2 μg/mouse). Inhalation anti-IgE treatment occurred 1 hour prior to OVA inhalation challenge. $p < 0.0001$ for overall difference between groups by ANOVA. Only differences between nonsensitized and sensitized groups different by subtest ($p < 0.0001$). Data are mean ± standard deviation. PMN, polymorphonuclear leukocyte.

comparable in magnitude to the nonsensitized wild-type mice and significantly less than the sensitized wild-type group (Fig. 2). Thus, overall it appeared that mast cells play a role in baseline airway responsiveness to methacholine and may serve to modulate airway responsiveness due to allergic inflammation. No such similar claim could be made for the removal of IgE. The removal of IgE had no negative impact on allergic inflammation or airway responsiveness. One possible explanation, however unlikely, was that small amounts of antigen-specific IgE were produced and bound to basophils despite anti-IgE treatment prior to sensitization. Crosslinking of basophils after aerosolized OVA challenge could result

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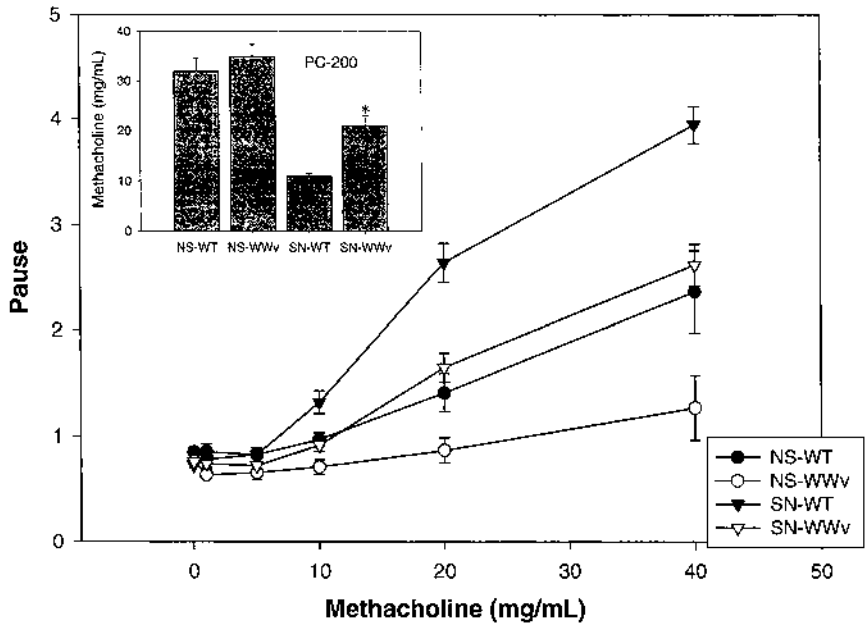

Figure 2 Mast cell deficiency (W/Wv) resulted in a baseline shift in airway responsiveness in nonsensitized mice, lowering the absolute responsiveness of sensitized W/Wv mice to that of nonsensitized wild-type mice. Sensitization and pretreatment as described in Figure 1. N as per Table 1. Twenty-four hours after the seventh OVA challenge, conscious mice were placed in a whole body plethysmograph and exposed to increasing concentrations of aerosolized methacholine for 1 minute. Pause [expiratory time/relaxation time −1], a measurement of airflow obstruction, was averaged for 4 minutes after each methacholine challenge. Data are expressed as mean ± standard error at each methacholine concentration. (Inset) The effect of mast cell deficiency on PC200. PC200 data are the mean ± standard error of the methacholine concentration causing a doubling of Pause. Group abbreviations: nonsensitized, wild-type (NS-WT); nonsensitized, mast cell–deficient (NS-W/Wv); sensitized, wild-type (SN-WT); sensitized, mast cell–deficient (SN-W/Wv). SN-W/Wv group is different from all other groups by Dunnett's test.

in inflammation since it has been estimated that 10% receptor occupancy is sufficient to trigger degranulation (42). However, there is no evidence to support this finding in our experiment. It is now clear that although we likely succeeded in eliminating IgE and mast cells, we did not eliminate IgG1 that may have contributed to our inability to find differences between treatment groups. Despite these reservations, other studies, as cited above, have reported that the lack of all immunoglobulins, the IgE high-affinity receptor, or mast cells does not result in the

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inability of actively sensitized and challenged mice to develop allergic inflammation, anaphylaxis, and, possibly, airway reactivity and airflow obstruction.

IV. Evidence for the Involvement of Mast Cells and IgE in Murine Asthma Models

Although mast cells and immunoglobulins may not be required in certain mouse models of asthma, multiple investigators have shown that under specific conditions they can contribute to allergic inflammation and lung function changes. Martin et al. (20) first demonstrated the importance of mast cells in airway reactivity by activating mast cells with a crosslinking anti-IgE antibody. In nonsensitized wild-type mice, crosslinking mast cells with anti-IgE resulted in increased airway hyperresponsiveness to intravenous methacholine challenge compared to saline-treated controls. However, surprisingly the hyperresponsiveness occurred only with methacholine challenge and not 5-HT. A direct role for mast cells was established by repeating the experiment using two strains of mast cell–deficient mice (W/Wv and Sl/Sl^{d}) and showing that airway reactivity was not increased in these strains. Bone marrow reconstitution of the mast cells in these deficient strains resulted in restoration of hyperresponsiveness.

Kung et al. (43) showed that W/Wv mice had an attenuated (~40%) inflammatory cell influx compared to congenic controls after systemic OVA sensitization followed by aerosolized OVA challenge, in contrast to the studies discussed in previous sections indicating that lack of involvement of mast cells in allergic inflammation. The authors reported decreased BAL and lung tissue eosinophils and decreased lung eosinophil peroxidase activity without an effect on serum IgE and IgG levels in the W/Wv mice compared to the sensitized controls. The critical role of mast cells in this response was demonstrated by showing that attenuation of allergic inflammation in W/Wv mice was fully reversed after adoptive transfer of bone marrow–derived mast cells into W/Wv mice 4–5 weeks prior to OVA sensitization. In a similar study, Kobayashi et al. (44) found that W/Wv mice had decreased airway responsiveness and a nonsignificant decrease in BAL eosinophilia. The apparent discrepancy between these two studies and those presented previously may have resulted from a difference in the severity of the inflammatory response in the different groups of studies (see discussion below).

In contrast to the Miyajima et al. (34) report of FcεRI-independent induction of anaphylaxis, Dombrowicz et al. (45) showed that FcεRI is requisite for the induction of anaphylaxis. Lack of FcεRI did not affect mast cell development, nor did it affect the half-life of IgE. FcεRI receptor–deficient mice were protected from anaphylaxis and had no tachycardia, body temperature drop, or Evans Blue

dye extravasation, three indicators of systemic anaphylaxis, when IgE was administered 24 hours before an anti-DNP IgE challenge (passive sensitization model). Wild-type control mice had obvious changes in these three parameters and were prostrate after anti-DNP IgE administration. This study indicates that an important role for FcεRI in systemic anaphylaxis can be shown in a passive sensitization model where the role of IgG1 is excluded or greatly minimized.

Several other experiments using passive sensitization have demonstrated a specific role of IgE in airway reactivity and allergic inflammation. Naïve BALB/c and SJL mice were passively sensitized with anti-OVA IgE and then challenged twice with aerosolized OVA (27). Eosinophils were increased in the BAL and tissue, and airway reactivity was increased compared to nonsensitized but OVA challenged mice. Similar responses were observed in mice challenged with anti-OVA IgG1 but not with IgG2a or IgG3 anti-OVA isotypes. Hamelmann et al. (46) extended this finding demonstrating that anti-OVA IgE caused airway reactivity in normal but not in athymic mice. Airway reactivity could be reinstated in the athymic mice if they received anti-OVA IgE plus IL-5. The authors concluded that both antigen-specific IgE and IL-5 were required to produce increased airway reactivity after antigen challenge in this passive sensitization model.

A role for IgE was also demonstrated in a strain of mice that produce large amounts of IgE in comparison to BALB/c mice (47). After systemic sensitization and multiple OVA aerosol challenges, only the high IgE–producing strain were hyperresponsive to both intravenous 5-HT or inhaled methacholine. In the high IgE–producing strain, there was also greater infiltration of eosinophils into the bronchial respiratory epithelium. Airway hyperresponsiveness, only observed in the high IgE producers, was suppressed with an anti IL-5 antibody, again indicating a role for both high IgE titers and IL-5.

Noncrosslinking anti-IgE antibodies have also been used to assess the role of IgE in murine models of asthma. In one study, a single dose administration of an anti-IgE antibody 6 hours prior to antigen challenge significantly lowered circulating levels of IgE and reduced eosinophil cell counts in the BAL (10). Secretion of IL-4 and IL-5 from pulmonary lymphocytes isolated from these sensitized mice was also lower. Heusser et al. (9) followed up these results and showed that inhibition of IgE with anti-IgE antibody in OVA-immunized mice reduced the acute bronchoconstriction that occurs following antigen challenge. Haile et al. (48) performed a similar antibody experiment using high IgE–producing mice. The same noncrosslinking anti-IgE antibody, when administered prior to antigen challenge, reduced BAL and tissue eosinophils, recruitment of IgE-bearing basophils, mucous metaplasia and acute antigen-induced bronchoconstriction, and nonspecific airway hyperresponsiveness. These studies demonstrated a significant role of IgE in the induction of allergic pulmonary inflammation in mice. However, in the study by Coyle et al. (10), all of the antibody

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effects could be reproduced by blocking CD23, the low-affinity receptor in CD23-deficient mice, suggesting that the effects observed with this noncrosslinking antibody were not due to blocking the high-affinity receptor, FcεRI.

V. Study 2: The Role of IgE in Murine Asthma Determined by the Model

It is apparent that the conclusions attained from studies with murine asthma models cited in the literature often conflict with one another concerning the importance of IgE. Faced with this we designed experiments to determine whether the role of IgE depends upon the model utilized. Our hypothesis was that sensitization strategies using a potent adjuvant may induce redundant mechanisms that in turn induce an asthma-like phenotype. These could include the induction of IgG1 or a Th2 lymphocyte response. To test this hypothesis, we utilized three models: a model using only aerosolized dust mite, a model combining aerosolized dust mite and respiratory syncytial virus (RSV) infection, and a model using systemic sensitization (intraperitoneal) with dust mite allergen adsorbed to the adjuvant alum. Prior to switching to dust mite antigen, we attempted to use ovalbumin to sensitize and challenge mice using only the inhalation route but were unable to induce inflammation even with numerous aerosol challenges given over a period of 6 months (unpublished data). The dust mite allergen *Dermatophagoides pteronyssinus* was chosen because the immunodominant protein Der p 1 is one of the major inducers of allergic disease (49). To further enhance the allergic immune response to aerosolized dust mite allergen, live RSV was co-administered. RSV infection has been reported to exacerbate airway inflammation in asthmatics (50–52) and is thought to be an important risk factor for the development of asthma in children (53,54). Furthermore, human RSV infection in mice can enhance airway sensitization to allergen and exacerbate the induced asthmatic phenotype in mice (55). After sensitization, all models used the same protocol for subsequent allergen challenge by aerosol exposure. After establishment of these three models, the role of IgE was assessed using and anti-IgE monoclonal antibody.

Use of only aerosolized dust mite antigen to both sensitize and challenge mice resulted in mild allergic pulmonary inflammation (data not shown). Both the IP/aero and RSV/aero models produced significant eosinophilic and lymphocytic pulmonary inflammation and a similar degree of mucosal pathology and thickening (Figs. 3 and 4). Thus, concomitant self-limiting viral infection had an enhancing or adjuvant effect similar to systemic immunization with alum.

Treatment with anti-IgE antibody reduced both serum and BAL fluid IgE by greater than a factor of 10 in both the IP/aero and RSV/aero groups compared to the non–IgE-depleted controls (Fig. 5). Although total IgE was reduced to equivalent low levels in all animals treated with the anti-IgE antibody, pulmonary

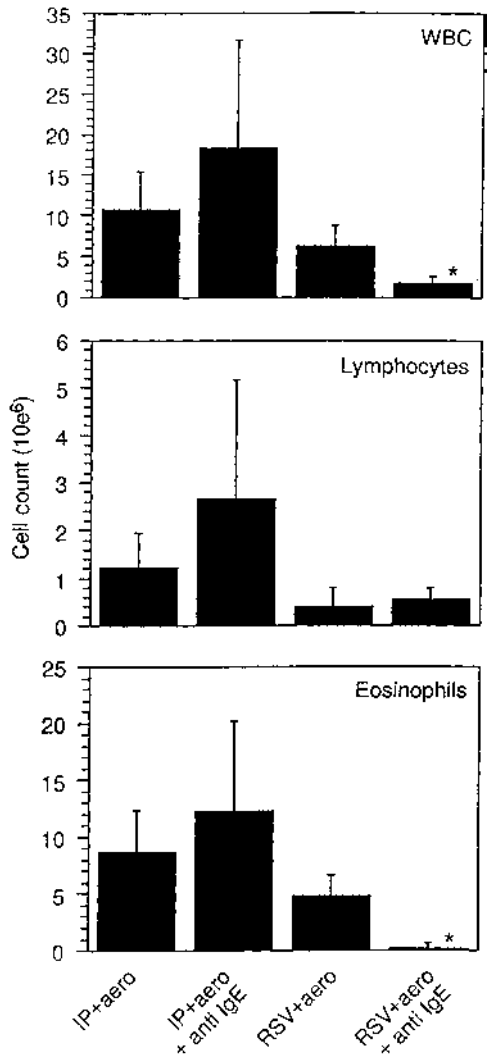

Figure 3 Anti-IgE treatment significantly reduced the number of white blood cell (WBC) eosinophils in the BAL of the RSV/aero group ($p < 0.05$) but had no effect on lymphocytes or in any cellular component of the IP/aero group. BAL was collected after aerosol challenges with dustmite antigen in mice previously sensitized to this antigen. Groups of mice were treated with the anti-IgE antibody or were untreated ($n = 6$/group). Data expressed as mean ± standard error and subtested with Duncan's multiple range test.

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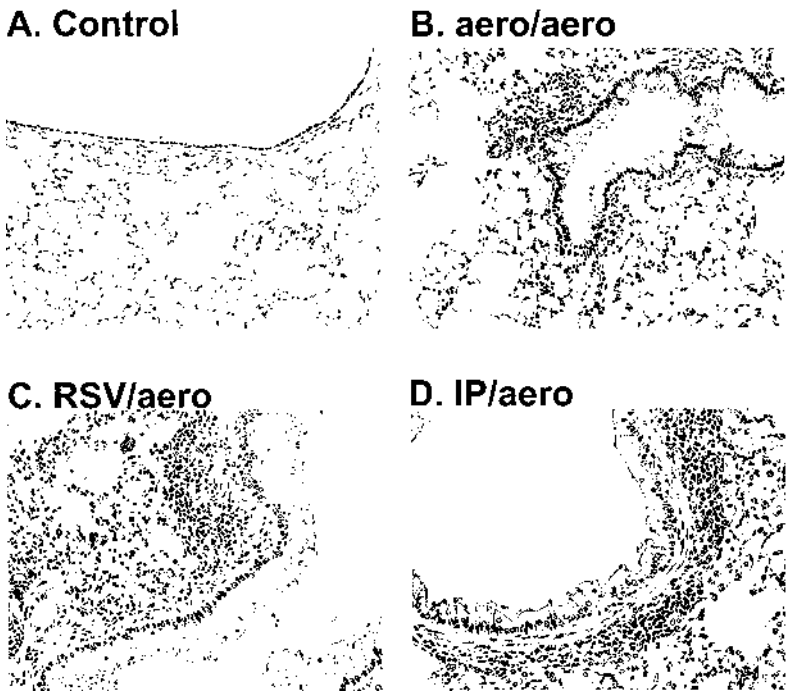

Figure 4 Anti-IgE demonstrated an ameliorative effect on both inhibiting eosinophilic inflammation and on decreasing mucosal epithelial hypertrophy in the RSV/aero-treated mice. Representative histological sections of main intrapulmonary lobar bronchi stained with routine hematoxylin and eosin (H&E) or with Alcian blue-PAS dye are shown from a control animal (A and B), an untreated animal in the RSV/aero group (C and D), and an anti-IgE–treated animal in the RSV/aero group (E and F). The H&E sections (A, C, and E) are magnified 400×, while the Alcian blue-PAS sections (B, D, and F) are magnified 800×. Anti-IgE treatment had no effect on the inflammation, mucosal height, or relative mucus production in the IP/aero group (data not shown).

inflammation was reduced only in mice sensitized by aerosol exposure with or without concomitant RSV infection. Anti-IgE treatment in the RSV/aerosol dust mite–sensitized group reduced the mean number of BAL eosinophils by a factor of 10. Since anti-IgE treatment had a specific effect on infiltration of eosinophils, we evaluated whether depletion of IgE had an ameliorative effect on large airway mucosal lesions. We quantitated the degree of large airway mucosal epithelial cell hypertrophy and mucosal thickening by determining the mean height of the mucosal epithelium in the primary lobar bronchi as measured by the distance from the mucosal basement membrane to the lumenal surface. In the RSV/aero

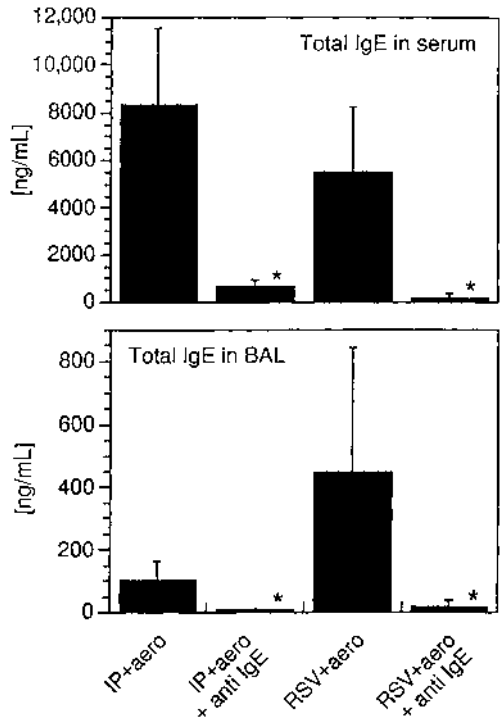

Figure 5 Anti-IgE treatment significantly reduced the IgE levels in the serum and BAL in all treated groups. The IP/aero group mice were sensitized to dustmite antigen (1 μg and 1 mg ALUM). The RSV/aero mice received $10e^6$ pfu of live virus intranasally on days 0 and 14 and received aerosolized antigen challenge on days 1 through 7. All groups of mice then received aerosol challenge from days 15–21. Anti-IgE antibody (100 μg) was administered to some of the mice ($n = 6$/group) from the IP/aero or the RSV/aero groups one day prior to and on the day of antigen administration.

animals the thickness of the mucosa in lobar bronchi was reduced from 38 μm ± 2.6 in untreated animals to 21 μm ± 2.7 in anti-IgE treated animals ($p < 0.05$) compared to the mean height of the mucosa in the main lobar bronchi of naïve controls, which was 14 μm ± 2.9. This effect of anti-IgE treatment on mucosal hypertrophy and thickness was accompanied by a reduction in mucus, as can be seen in Figure 4, which shows lobar bronchi sections stained with Alcian blue-PAS.

In contrast, IgE depletion in animals sensitized to dust mite allergen by systemic exposure with adjuvant had no effect on pulmonary inflammation (mean numbers of BAL leukocytes, eosinophils, or lymphocytes) or mucosal lesions (Fig. 4). The lack of efficacy of anti-IgE therapy in the systemic sensitization

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model (IP/aero) suggests that other mediators were induced that could drive allergic pulmonary eosinophilic inflammation. These alternative mechanisms could involve chemokines (6), interleukins, or allergen-specific IgG1.

These results demonstrate that by using sensitization protocol (RSV/aero) more similar to natural allergen sensitization and exposure as occurs in humans, one can generate IgE-dependent allergic pulmonary inflammation in mice. The study also demonstrates that the mechanisms and pathogenesis of allergic inflammation in murine models can differ significantly between models that differ in sensitization strategies. It is apparent that in the systemic sensitization model both IgE and other, apparently redundant, mediators are induced.

VI. Summary and Conclusions

At first glance, obvious contradictions in the data might question the usefulness of animal models in evaluating the role of immunoglobulins and mast cells in human asthma. However, with careful examination of these studies taking into account differences between human and murine biology, evaluation of the differences between murine asthma models, coupled with the perspective gained by recent clinical studies using an anti-IgE antibody, a new understanding emerges.

Several of the discussed studies similarly concluded that anaphylaxis and allergic airway inflammation could be induced in the absence of IgE, mast cells, or the FcεRI receptor in the mouse. A caveat to this interpretation is that the mouse has at least one redundant mechanism that does not exist in humans. In the mouse, mast cell activation induced by the interaction of IgE and mast cell FcεRI receptor is well characterized. However, in the mouse the interaction of IgG1 and mast cell FcγRIII receptor can also induce mast cell activation and degranulation (56). Indeed, loss of the FcεRI α chain results in an enhancement of FcγRIII mast cell degranulation. It is clear that the immunization strategies described in many of the above reports induce a robust IgG1 antiallergen response in our hands, and thus the relative role of IgG1-mast cell FcRIII receptor interaction should not be overlooked in these models. Furthermore, in studies with mast cell–deficient mice, IgE can interact with basophils and eosinophils and the degranulation of these cells may be capable of inducing allergic pulmonary disease. On the other hand, when both mast cells and IgE are removed as in our studies, or when no immunoglobulins are present as in B-cell–deficient mice as well as in the Th2 lymphocyte transfer studies, allergic inflammation and airway hyperresponsiveness can be produced.

Despite these caveats, the data obtained from certain murine asthma models demonstrate significant modulatory effects of both mast cells and IgE on allergic inflammation. Notably, these models include mice specifically bred to produce

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high-IgE, passive IgE sensitization models, and models that do not generate severe airway inflammation either by eliminating adjuvant-enhanced systemic immunizations or by using small numbers of aerosol challenges. In the case of high IgE–producing mice or with passive IgE models, the role of IgG1 is either greatly reduced or eliminated. In models without intense systemic sensitization with adjuvant (57) or with few antigen inhalation challenges (44,58), the balance between IgE and IgG1 may be tipped in favor of IgE, thus allowing the role of IgE to be elucidated. Alternatively, these less intense sensitization and challenge models may decrease the impact of mediators from other infiltrating cells, notably lymphocytes and eosinophils relative to mediators expressed by mast cells. However, even in these models, where the influence of IgE and mast cells are greatest, inflammation was significantly attenuated but rarely was it ablated, suggesting that additional mechanisms are responsible for the inflammation.

Mast cells can, however, be shown to contribute to airway responsiveness and airflow obstruction. In several published studies (33,44), as well as by our own data, allergic inflammation was not suppressed in sensitized W/Wv mice, yet there was an attenuated response to nonspecific and specific airway challenge. Possibly this is due to an effect of inflammation amplifying cholinergic responsiveness since it was not true of 5-HT challenge (20) and baseline methacholine reactivity in naïve mice was significantly altered in our studies (41). This amplification may also occur by redundant mechanisms that become important with more intense antigenic stimulation, as discussed above. This is best exemplified by a study by Kobayashi et al. (44) in which airway responsiveness was attenuated when W/Wv mice received three antigen inhalation challenges after sensitization. However, when the same sensitization protocol was followed by multiple antigen challenges per day over several days, there was no difference in airway responsiveness between control and W/Wv mice.

These conclusions beg the question of how these murine models of asthma compare to the clinical situation. Several clinical trials have now been carried out using a monoclonal anti-IgE antibody that binds free IgE without crosslinking FcεRI receptors on mast cells. Results from clinical bronchoprovocation studies (59,60), discussed in detail in other chapters in this book, indicate that systemic anti-IgE is effective in reducing antigen-provoked early and late asthmatic responses, airway hyperresponsiveness, and eosinophil accumulation in induced-sputum. Furthermore, in clinical trials of asthmatics, Milgrom et al. (61) demonstrated that anti-IgE therapy reduced symptom scores and exacerbations of asthma and allowed a greater number of subjects to decrease or discontinue their use of corticosteroids than was observed in the placebo-treated group. Of particular note is the trial design that used patients optimally treated with corticosteroids and β-agonists prior to and during anti-IgE treatment, therefore biasing the trail against finding a positive result. Anti-IgE was clearly efficacious in these already well-controlled patients and therefore will be a valuable asset in the treatment

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of asthma. As seen in certain mouse models, some patients, however, did not respond completely to anti-IgE therapy. With this perspective, the relevance of different mouse models may be clearer.

In the clinical situation, as demonstrated by the effect of anti-IgE, IgE (and by inference mast cells) plays a role in asthma, probably both as a trigger for initiating the allergic cascade as well as an effector of response. Similarly, in the mouse, when the redundant immunoglobulin IgG1 is removed or reduced, IgE and mast cells can be shown to modulate allergic inflammatory responses. However, in mice and possibly in humans, removal of IgE is not necessarily curative. Data from the murine models suggest that Th2-cell–derived cytokines can cause a similar inflammatory response in the absence of immunoglobulin. Whether these apparently IgE-independent effects occur in humans is currently unknown.

Asthma is a disease defined by phenotype, not mechanism. Overall, animal models suggest this phenotype may arise by heterogeneous pathways in which there may be separate, redundant, or multiple mechanisms responsible for the clinical manifestation of the disease. The responsiveness to different therapies in mice appears to depend upon the relative prevalence of other redundant mediators as produced by different sensitization and challenge protocols. Careful examination and improvement in our understanding of animal models of asthma may eventually help us unravel and treat this complex disease.

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References

1. Gavett SH, et al. Depletion of murine CD4+ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. Am J Respir Cell Mol Biol 1994; 10(6):587–593.
2. Zuany-Amorim C, et al. Requirement for gammadelta T cells in allergic airway inflammation. Science 1998; 280(5367):1265–1267.
3. Padrid PA, et al. CTLA4Ig inhibits airway eosinophilia and hyperresponsiveness by regulating the development of Th1/Th2 subsets in a murine model of asthma. Am J Respir Cell Mol Biol 1998; 18(4):453–462.
4. Van Oosterhout AJ, et al. Murine CTLA4-IgG treatment inhibits airway eosinophilia and hyperresponsiveness and attenuates IgE upregulation in a murine model of allergic asthma. Am J Respir Cell Mol Biol 1997; 17(3):386–392.

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5. Pauwels RA, Brusselle GJ, Kips JC. Cytokine manipulation in animal models of asthma. Am J Respir Crit Care Med 1997; 156(4 Pt 2):S78–81.
6. Gonzalo JA, et al. Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and adhesion receptors. J Clin Invest 1996; 98(10):2332–2345.
7. Gonzalo JA, et al. The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. J Exp Med 1998; 188(1): 157–167.
8. Rothenberg ME, et al. Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. J Exp Med 1997; 185(4):785–790.
9. Heusser CH, et al. Demonstration of the therapeutic potential of non-anaphylactogenic anti-IgE antibodies in murine models of skin reaction, lung function and inflammation. Int Arch Allergy Immunol 1997; 113(1–3):231–235.
10. Coyle AJ, et al. Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. J Exp Med 1996; 183(4):1303–1310.
11. Chin JE, et al. Airway recruitment of leukocytes in mice is dependent on alpha4-integrins and vascular cell adhesion molecule-1. Am J Physiol 1997; 272(2 Pt 1): L219–229.
12. Chin JE, et al. Involvement of intercellular adhesion molecule-1 in the antigen-induced infiltration of eosinophils and lymphocytes into the airways in a murine model of pulmonary inflammation. Am J Respir Cell Mol Biol 1998; 18(2):158–167.
13. Hatfield CA, et al. Intercellular adhesion molecule-1-deficient mice have antibody responses but impaired leukocyte recruitment. Am J Physiol 1997; 273(3 Pt 1): L513–523.
14. Henderson WR, Jr., et al. Blockade of CD49d (alpha4 integrin) on intrapulmonary but not circulating leukocytes inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma. J Clin Invest 1997; 100(12):3083–3092.
15. Wolyniec WW, et al., Reduction of antigen-induced airway hyperreactivity and eosinophilia in ICAM-1-deficient mice. Am J Respir Cell Mol Biol 1998; 18(6):777–785.
16. De Sanctis GT, et al. T-lymphocytes regulate genetically determined airway hyperresponsiveness in mice [see comments]. Nat Med 1997; 3(4):460–462.
17. Stelts D, et al. Eosinophils retain their granule major basic protein in a murine model of allergic pulmonary inflammation. Am J Respir Cell Mol Biol 1998; 18(4):463–470.
18. Gleich GJ, Kita H. Bronchial asthma: lessons from murine models [comment]. Proc Natl Acad Sci USA 1997; 94(6):2101–2102.
19. Burrows B, et al. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989; 320(5):271–277.
20. Martin TR, et al. Mast cells contribute to the changes in heart rate, but not hypotension or death, associated with active anaphylaxis in mice. J Immunol 1993; 151(1): 367–376.
21. Naclerio RM, et al. Intranasal steroids inhibit seasonal increases in ragweed-specific immunoglobulin E antibodies. J Allergy Clin Immunol 1993; 92(5):717–721.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

22. Sears MR, et al. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. N Engl J Med 1991; 325(15):1067–1071.
23. Serafin WE, Austen KF. Mediators of immediate hypersensitivity reactions. N Engl J Med 1987; 317(1):30–34.
24. Sihra BS, et al. Expression of high-affinity IgE receptors (Fc epsilon RI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. J Allergy Clin Immunol 1997; 99(5): 699–706.
25. Gessner JE, et al. The IgG Fc receptor family. Ann Hematol 1998; 76(6):231–248.
26. Ra C, Jouvin MH, Kinet JP. Complete structure of the mouse mast cell receptor for IgE (Fc epsilon RI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. J Biol Chem 1989; 264(26):15323–15327.
27. Oshiba A, et al. Passive transfer of immediate hypersensitivity and airway hyperresponsiveness by allergen-specific immunoglobulin (Ig) E and IgG1 in mice. J Clin Invest 1996; 97(6):1398–1408.
28. Takeishi T, et al. Differences in the expression of the cardiopulmonary alterations associated with anti-immunoglobulin E-induced or active anaphylaxis in mast cell-deficient and normal mice. Mast cells are not required for the cardiopulmonary changes associated with certain fatal anaphylactic responses. J Clin Invest 1991; 88(2):598–608.
29. Brusselle GG, et al. Attenuation of allergic airway inflammation in IL-4 deficient mice [see comments]. Clin Exp Allergy 1994; 24(1):73–80.
30. Takeda K, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. J Exp Med 1997; 186(3):449–454.
31. Oettgen HC, et al. Active anaphylaxis in IgE-deficient mice. Nature 1994; 370(6488):367–370.
32. Mehlhop PD, et al. Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma [see comments]. Proc Natl Acad Sci USA 1997; 94(4):1344–1349.
33. Hamelmann E, et al. Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness—a murine model. Allergy 1999; 54(4):297–305.
34. Miyajima I, et al. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. J Clin Invest 1997; 99(5):901–914.
35. Kitamura D, et al. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene. Nature 1991; 350(6317):423–426.
36. Hamelmann E, et al. Allergic airway sensitization induces T cell activation but not airway hyperresponsiveness in B cell-deficient mice. Proc Natl Acad Sci USA 1997; 94(4):1350–1355.
37. Korsgren M, et al. Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. J Exp Med 1997; 185(5):885–892.
38. MacLean JA, et al. Antigen-induced airway hyperresponsiveness, pulmonary eosinophilia, and chemokine expression in B cell-deficient mice. Am J Respir Cell Mol Biol 1999; 20(3):379–387.

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39. Cohn L, et al. Induction of airway mucus production by T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus production. J Exp Med 1997; 186(10):1737–1747.
40. Cohn L, Tepper JS, Bottomly K. IL-4-independent induction of airway hyperresponsiveness by Th2, but not Th1, cells. J Immunol 1998; 161(8):3813–3816.
41. Tepper J, et al. The role of mast cells and IgE in murine asthma (abstr). Asthma 1995; 95.
42. Kagey-Sobotka A, MacGlashan DW, Lichtenstein LM. Role of receptor aggregation in triggering IgE-mediated reactions. Fed Proc 1982; 41(1):12–16.
43. Kung TT, et al. Mast cells modulate allergic pulmonary eosinophilia in mice. Am J Respir Cell Mol Biol 1995; 12(4):404–409.
44. Kobayashi T, et al. An essential role of mast cells in the development of airway hyperresponsiveness in a murine asthma model. J Immunol 2000; 164(7):3855–3861.
45. Dombrowicz D, et al. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. Cell 1993; 75(5):969–976.
46. Hamelmann E, et al. Allergen-specific IgE and IL-5 are essential for the development of airway hyperresponsiveness. Am J Respir Cell Mol Biol 1997; 16(6):674–682.
47. Eum SY, et al. Eosinophil recruitment into the respiratory epithelium following antigenic challenge in hyper-IgE mice is accompanied by interleukin 5-dependent bronchial hyperresponsiveness. Proc Natl Acad Sci USA 1995; 92(26):12290–12294.
48. Haile S, et al. Suppression of immediate and late responses to antigen by a non-anaphylactogenic anti-IgE antibody in a murine model of asthma. Eur Respir J 1999; 13(5):961–969.
49. Shakib F, Schulz O, Sewell H. A mite subversive: cleavage of CD23 and CD25 by Der p 1 enhances allergenicity. Immunol Today 1998; 19(7):313–316.
50. Minor TE, et al. Viruses as precipitants of asthmatic attacks in children. JAMA 1974; 227(3):292–298.
51. Beasley R, et al. Viral respiratory tract infection and exacerbations of asthma in adult patients. Thorax 1988; 43(9):679–683.
52. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults [see comments]. Br Med J 1993; 307(6910):982–986.
53. Sigurs N, et al. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. Pediatrics 1995; 95(4):500–550.
54. Strannegard O, et al. Association between pronounced IgA response in RSV bronchiolitis and development of allergic sensitization. Pediatr Allergy Immunol 1997; 8(1): 1–6.
55. Schwarze J, et al. Respiratory syncytial virus infection results in airway hyperresponsiveness and enhanced airway sensitization to allergen. J Clin Invest 1997; 100(1): 226–233.
56. Dombrowicz D, et al. Absence of Fc epsilonRI alpha chain results in upregulation of Fc gammaRIII-dependent mast cell degranulation and anaphylaxis. Evidence of competition between Fc epsilonRI and Fc gammaRIII for limiting amounts of FcR beta and gamma chains. J Clin Invest 1997; 99(5):915–925.
57. Williams CM, Galli SJ. Mast cells can amplify airway reactivity and features of

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chronic inflammation in an asthma model in mice. J Exp Med 2000; 192(3):455–462.
58. Kung TT, et al. Characterization of a murine model of allergic pulmonary inflammation. Int Arch Allergy Immunol 1994; 105(1):83–90.
59. Boulet LP, et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response [see comments]. Am J Respir Crit Care Med 1997; 155(6): 1835–1840.
60. Fahy JV, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects [see comments]. Am J Respir Crit Care Med 1997; 155(6):1828–1834.
61. Milgrom H, et al. Treatment of allergic asthma with monoclonal anti-IgE antibody. rhuMAb-E25 Study Group [see comments]. N Engl J Med 1999; 341(26):1966–1973.

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8

Role of IgE in Asthma

Pathogenesis and Novel Therapeutic Strategies

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I. Introduction

The prevalence and severity of asthma and other allergic diseases are increasing worldwide, reflected by an increase in asthma-related symptoms, medication usage, and hospital admissions for asthma (1,2).

Asthma is closely associated with atopy, characterized by elevated circulating allergen-specific IgE and positive skinprick responses to allergen testing. Recent epidemiological and immunopathological studies have increased our understanding of the involvement of allergen sensitization and IgE-mediated mechanisms in the genesis and persistence of chronic airway inflammation in asthma. Data also suggest that local IgE-mediated mechanisms may contribute to the chronic airway inflammation of ''intrinsic,'' or nonatopic asthma, where allergen sensitization has not been identified.

In this chapter we provide an overview of the current cellular and molecular mechanisms involved in airway inflammation, in particular the involvement of IgE-mediated mechanisms, and the application of novel therapeutic agents to suppress ongoing airway inflammatory responses in asthma.

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II. Asthma Epidemiology: The Significance of Allergen-Specific IgE

Approximately 80% of asthmatics are sensitized to specific environmental allergens, such as house dust mites, pollens, and animal danders. Although atopy is the single strongest risk factor for asthma and increases the risk of asthma 10- to 20-fold, only about one fifth of atopic subjects develop clinical asthma. Strong epidemiological associations have been shown between asthma and atopy, as assessed by total serum IgE or positive skinprick tests in childhood (3) and in adults (4). Studies have confirmed associations between the level of indoor allergen exposure, including house dust-mite allergen (*Der p*-1) and cat allergen (*Fel d* 1) and the degree of IgE sensitization, serum IgE levels, and the frequency and severity of asthma symptoms (5–8) and airway hyperresponsiveness (4,9–11). Domestic exposure in early life to *Der p* 1 levels of greater than 2 μg per gram of dust has been shown to significantly increase the risk of initial allergen sensitization and the development of asthma, and exposure to levels over 10 μg per gram of dust increases the risk of developing acute exacerbations of preexisting asthma (12–14). Exposure to *Alternaria* allergens has been linked to acute life-threatening attacks in asthmatics (15), and other allergens, including animal dander (16), insect dust, grass pollen (17), and molds (18), have also been implicated in asthma exacerbations.

III. Pathological Features of Asthma

Asthma is characterized by variable damage to the bronchial epithelium and increased numbers of airway mucosal and submucosal inflammatory cells, including eosinophils, mast cells, and T lymphocytes (19–21). Other prominent features of asthma include hypertrophy and hyperplasia of airway smooth muscle, basement membrane thickening, mucosal edema and excessive secretion of mucus, all of which contribute to airway narrowing (22).

In severe exacerbations both large and small airways show gross damage and shedding of the epithelium, and the airways may become occluded by inspissated mucus and cellular debris, which form tenacious plugs (Fig. 1). Studies

Figure 1 Characteristic pathological features of asthma: (a) Epithelial disruption, subbasement membrane collagen deposition and infiltration of the submucosa with inflammatory cells—specifically eosinophils, mast cells, T cells, and tissue macrophages; (b) Epithelial metaplasia with an increase in goblet cells. Chronic asthma is also characterized by hyperplasia of airway smooth muscle beneath the inflamed submucosa and the deposition of matrix proteins both in the submucosa and adventitia of the airway (H & E stain).

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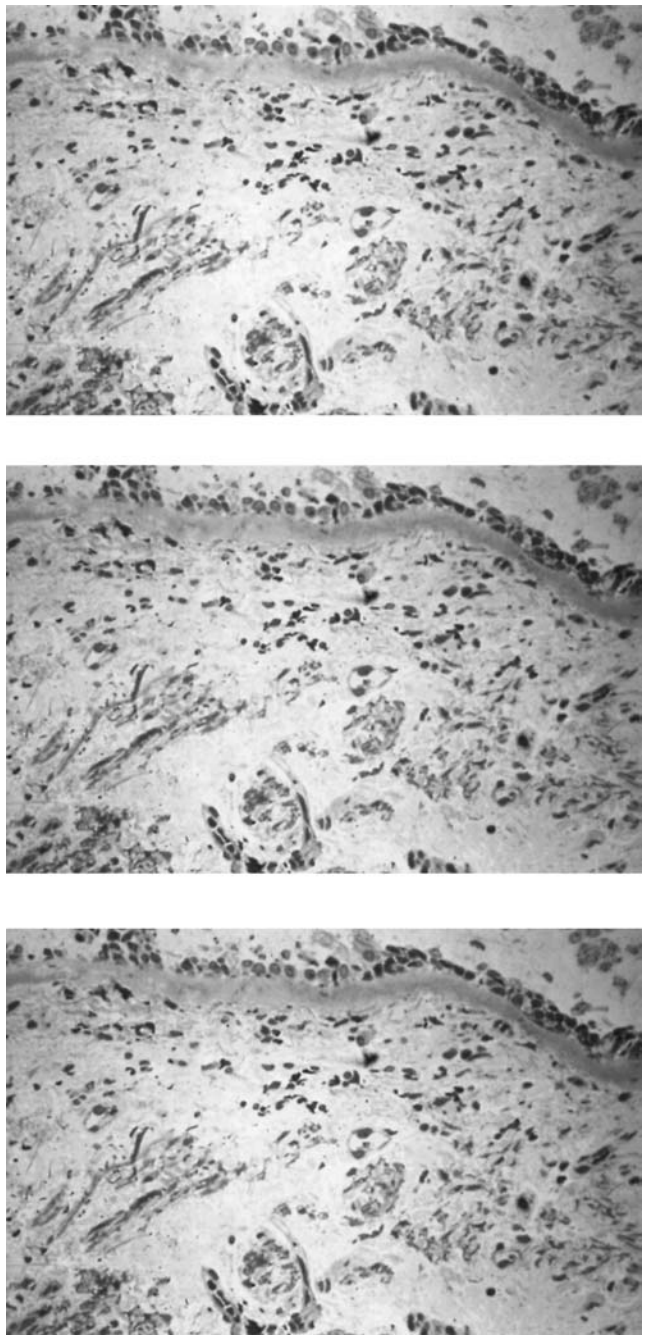

(a)

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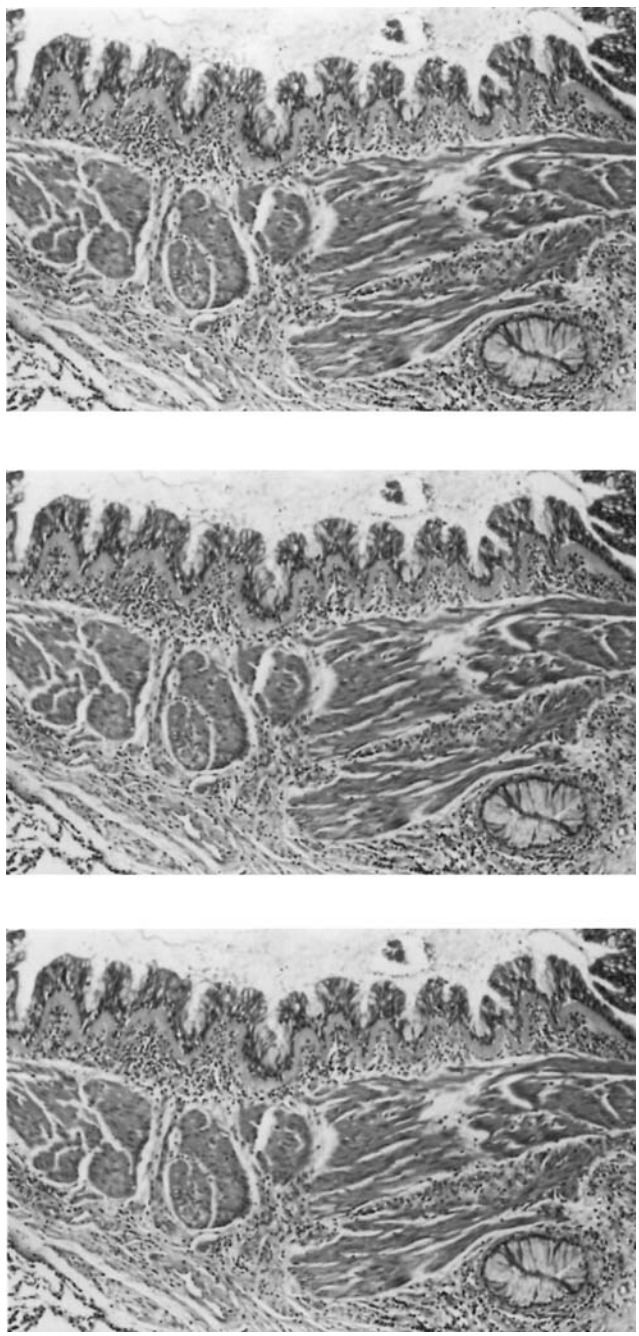

(b)

Figure 1 Continued

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suggest differences in the type of inflammation depending on the time-course of asthma exacerbation, with infiltration by eosinophils being noted in slow-onset fatal asthma and an excess of neutrophils being the prominent feature of sudden acute asthma deaths (23).

IV. Cellular Involvement in Asthma

Asthma has been traditionally viewed as a chronic inflammatory condition characterized by airway infiltration by activated mast cells and eosinophils, orchestrated by specific Th2-type T lymphocytes (24). However, neutrophils have recently been implicated in more severe forms of asthma, and it is also increasingly evident that the bronchial epithelium, endothelium, fibroblasts, and the extracellular matrix play a dynamic role in the airway inflammation of asthma (Fig. 2).

A. The Orchestrating Role of T Lymphocytes in Airway Inflammation

There is considerable evidence to support the view that the asthmatic airway inflammation is driven by the persistence of chronically activated T cells of a memory (CD 45 RO+) phenotype, which are sensitized to specific allergenic, occupational, or viral antigens, and localize to the airways after appropriate antigen exposure (25) or viral infection. This hypothesis is supported by observations in studies using broncho alveolar lavage (BAL) and bronchial biopsies from asthmatic subjects, where elevated numbers of circulating activated CD25+ CD4+ T cells are seen in acute severe asthma, which are closely correlated with the level of airflow obstruction (26). Analysis by immuno-histochemistry, in situ hybridization (ISH), and flow cytometry of bronchial biopsies and BAL from symptomatic asthmatics has also confirmed increased numbers of activated T cells (19,27). Of particular interest is the correlation shown between activated CD4+ lymphocytes, eosinophils, and cells positive for IL-5 mRNA in BAL, supporting the view that T cells regulate the accumulation and function of eosinophils in the airways in asthma by the release of interleukin-5 (IL-5) (28).

Studies support the view that CD4+ (Th2) and cytotoxic CD8+ (Tc) T cells are a major source of Th2-type cytokines in the airways in asthma (29–32). Co-localization studies of BAL and bronchial biopsy samples from both atopic and nonatopic asthmatics confirm that T cells are the predominant cells encoding mRNA for IL-3, IL-4, IL-5, IL-10, and GM-CSF. T cells are considered to play an important role in regulating IgE synthesis in asthma. This is mediated by the increased T-cell production of IL-4 and IL-13 and the accentuated expression of the accessory molecule CD40L on activated T cells in asthma, which binds to its ligand on B cell after B cell–T cell physical contact, delivering essen-

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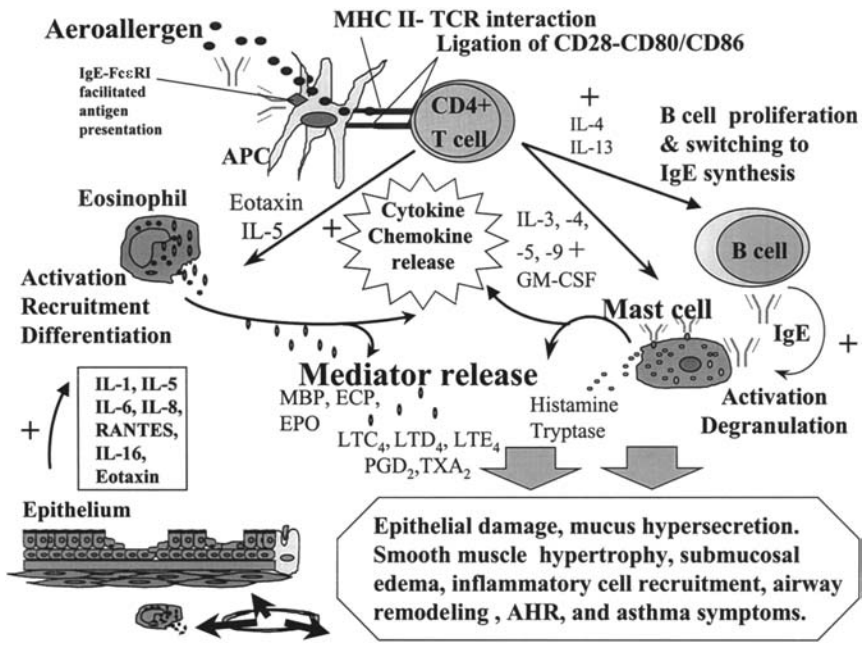

Figure 2 The cellular basis and cytokine network involved in asthma airway inflammation. Airway dendritic cells (antigen-presenting cells, APC) present allergen to resident T cells in the context of major histocompatibility molecule (MHC) II complex–T-cell receptor (TCR) ligation, and costimulatory signaling via the CD28-CD80/CD86 pathway resulting in the production of Th2-type cytokines and chemokines (IL-16, eotaxin, eotaxin-2). This promotes T-cell proliferation and differentiation to a Th2 phenotype (IL-4), the recruitment, activation, and survival of eosinophils (IL-5, eotaxin), and the switching of B cells to IgE synthesis (IL-4, IL-13). Mediators released by eosinophils [major basic protein (MBP); eosinophil cationic protein (ECP); eosinophil peroxidase; cysteinyl leukotrienes (LTC_4, LTD_4, LTE_4)], and mast cells [histamine; tryptase; prostaglandins (PGE_2); thromboxane A_2 (TXA_2)] result in airway inflammation and the clinical syndrome of asthma.

tial accessory signals required by B cells for clonal expansion, proliferation, and isotype class switching to the synthesis of IgE (20).

Therapeutic studies have demonstrated a role for T cells in asthma airway inflammation. Prednisolone treatment in corticosteroid-sensitive asthmatics (CSA) results in improved lung function, reduced airway hyperresponsiveness (AHR), accompanied by a reduction in eosinophil counts, and a reduction in the numbers of CD4+ T cells expressing mRNA encoding IL-3, IL-5, and granulocyte macrophage-colony stimulating factor (GM-CSF) but not IL-2, IL-4, or IFNγ

in BAL (33–35). However, a minority of subjects develop a severe form of asthma that is refractory to systemic corticosteroid treatment (corticosteroid-resistant asthma, CRA). Compared to CSAs these subjects have increased numbers of circulating CD4+ CD25+ T cells and in vitro peripheral blood mononuclear cell (PBMC) cultures from CRAs show ongoing mitogen-induced proliferation when cultured in the presence of dexamethasone (36). Bronchial biopsies from CRAs also show increased expression of IFNγ and persisting IL-4 and IL-5 mRNA expression, which is not reduced by steroid treatment (37).

B. The Effector Role of Eosinophils

Elevated eosinophil counts have consistently been shown in the airways in asthma both at baseline and after segmental allergen challenge, and eosinophil influx has been closely related to the late phase bronchoconstrictor response (21). Eosinophils have been closely associated with the degree of airway inflammation, epithelial damage, and disease severity in asthma as confirmed by eosinophil counts in the airway lumen, mucosal tissue, and induced sputum (38) IL-3, IL-5, and GM-CSF release has been shown to prolong airway eosinophil survival by inhibiting apoptosis and to prime eosinophils for mediator release (39).

A number of pro-inflammatory proteins (major basic protein, MBP; eosinophil cationic protein, ECP; eosinophil-derived neurotoxin; and eosinophil peroxidase, EPO) and lipid products (cysteinyl leukotrienes, LTC_4, LTD_4, LTE_4; prostaglandins, PGE_2; thromboxane TxB_2; 15-HETE; and platelet activating factor, PAF) released by degranulation of activated eosinophils have been shown to promote smooth muscle contraction, tissue edema, and mucus secretion and damage the epithelial integrity by disruption of epithelial desmosomes and tight junctions (22). Eosinophils have also been shown to synthesize a number of pro-inflammatory cytokines including IL-1, IL-3, IL-4, IL-5, IL-6, IL-16, TNF-α, GM-CSF, IFN-γ, and transforming growth factors (TGF-α and TGF-β) involved in allergic inflammation and the remodeling process in asthma (40).

The release of IL-5 and eotaxin in the airways in asthma has been closely associated with the bronchial infiltration and accumulation of eosinophils (41), by promoting the initial development of eosinophils from bone marrow progenitor cells that express CD34+ and IL-5 receptor-α (28,42–44). However, a recent study by Robinson et al. has shown increased numbers of CD34+/IL-5 receptor-α mRNA-positive cells in the bronchial tissue of asthmatic compared to nonasthmatic patients, suggesting that in situ eosinophil differentiation may occur in the bronchial mucosa in asthma in response to local IL-5 production (45).

A recent study in atopic asthma has confirmed increased expression of high-affinity IgE receptors (FcεR1) on eosinophils (80–91%) in BAL 24 hours after segmental allergen challenge compared to baseline (4%) (46), supporting the view that eosinophils may be involved in allergen presentation to T cells (47)

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and that cross-linkage of FcεRI receptors may result in eosinophil degranulation and the bronchial epithelial damage characteristic of asthma.

C. Mast Cells

Mast cells have long been viewed as having a pivotal role in the development of the EAR in asthma via the allergen-specific activation of IgE-bound FcεRI receptors on the cell surface (48). Receptor aggregation and signal transduction leads to the release of an array of preformed and newly synthesized pro-inflammatory mediators (histamine, heparin, tryptase; prostaglandins, PGD_2 and PGE_2; leukotriene C_4, LTC_4; and thromboxane A_2, TXA_2) with spasmogenic and vasoactive properties (49). Increased numbers of $FcεRI^+$ cells have been shown in atopic and nonatopic asthmatics compared with control subjects and are predominantly co-localized to mast cells, macrophages, and eosinophils (50). The increased numbers of mast cells in bronchial biopsies and elevated mediator release in BAL from atopic asthmatics have been shown to correlate inversely with forced expiratory volume in one second (FEV_1) and AHR, respectively (24,51). Mast cells produce an array of cytokines (IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, and RANTES) relevant to the EAR and LAR in asthma (52).

Although primarily viewed as an effector cell, mast cells are also involved in regulating allergic responses. In allergic responses, mast cells are noted to express increased levels of FcεRI and CD40L and produce IL-4 and IL-13 and can directly control the synthesis of IgE by B cells (53). In vitro studies have previously shown that mast cells can be induced to express CD40L and promote IgE synthesis by interaction with B cells in the presence of exogenous IL-4. Mast cells can also modulate T-cell activation and Th2-type differentiation by cytokine release and the ability to present antigen to T cells in a major histocompatibility complex (MHC)-II and co-stimulatory dependent fashion (54). Mast cell proteases such as tryptase can activate matrix metallo-proteases that stimulate the growth of fibroblasts and smooth muscle cells, supporting the view that mast cells may be involved in the airway remodeling of asthma (55).

D. Basophils

Basophils are thought to contribute significantly to allergic inflammation. A recent study by MacFarlane and coworkers demonstrated increased numbers of basophils in the airways of asthmatics compared to rhinitic or normal control subjects, both at baseline and after allergen challenge (56,57). This supports the results of Guo and coworkers, suggesting that the majority of IgE-bearing cells recruited to the airways 17–22 hours after allergen challenge were basophils (58). In vitro studies have shown that basophils can be activated by IgE-dependent mechanisms, with the release of a significant amount of IL-4 early after allergen

stimulation and IL-13 release at a later stage, which may play a role in promoting the differentiation of CD4+ T cells to a Th2 phenotype and the synthesis of IgE (59,60).

V. Mechanisms of Asthma Severity and Chronicity

Despite the considerable improvement in the understanding of asthma pathogenesis, the mechanisms that determine disease severity remain poorly understood. The degree of sputum, BAL, and blood eosinophilia and, to a lesser extent, levels of ECP in blood have been found to broadly relate to disease severity. T-cell activation and expression of Th2-type cytokines is found in all degrees of asthma, but, as discussed above, it is most prominent in severe disease that is poorly controlled by corticosteroids (61). Physiological indices of asthma severity such as methacholine responsiveness are related to the number and activity of eosinophils and activated CD4+ T cells in BAL from atopic asthmatics (62,63). Airway hyperresponsiveness but not symptom scores has been shown to be inversely related to the number of activated eosinophils and mast cells in bronchial biopsies from asthmatics not treated with corticosteroids (64). In asthmatics treated with inhaled corticosteroids, AHR, but not symptom score or lung function, is inversely related to the number of infiltrating mast cells, activated eosinophils, CD8+, and $CD45RO^+$ T cells in bronchial biopsies from these patients (63).

Recent studies by Wenzel and coworkers suggest that neutrophils may play an important role in the inflammatory response in more severe forms of asthma (65). This study showed that while mucosal eosinophil numbers may or may not be raised, a significantly higher neutrophil count is seen in bronchial and transbronchial biopsies of severe, corticosteroid-dependent asthmatics compared with moderate asthmatics and normal controls (65). Other studies have documented increased numbers of neutrophils in induced sputum in severe asthma (66), during exacerbations (67), and in the circulation post–allergen challenge (68). Postmortem studies have shown distinct pathological features in cases of sudden-onset asthma death, with a paucity of airway eosinophils but marked neutrophilic infiltration compared to cases of slow-onset asthma death (23,67), where eosinophilic airway infiltration, gross epithelial shedding, airway edema, and obstruction with inspissated mucus plug formation are prominent. Some studies have shown that corticosteroids can enhance neutrophil function through increased leukotriene and superoxide production and inhibition of apoptosis (69). Lipid-derived and mast cell–derived mediators remained elevated in severe asthmatics despite treatment with high-dose corticosteroids. It is possible that corticosteroids could reduce lymphocyte and eosinophil-mediated inflammation but could exacerbate the neutrophil-mediated inflammation in severe asthma (65).

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VI. IgE-Facilitated Antigen Presentation in Asthma

Antigen presentation of allergens to the immune system is central to the initiation and maintenance of airway inflammation in asthma. On exposure to antigen, T cells require the assistance of ''professional'' antigen-presenting cells (APCs), such as dendritic cells (DCs), tissue macrophages, or, in the case of secondary presentation B cells, for optimal activation (70). Dendritic cells are believed to be the main APCs in the airways, being strategically positioned in the bronchial epithelium for the uptake and presentation of allergen to T cells. Dendritic cell numbers are elevated in the mucosa of asthmatic individuals and are reduced by treatment with inhaled corticosteroids in association with improved disease control (71,72).

Emerging evidence suggests that IgE may potentiate allergen-specific responses by binding to high- and low-affinity IgE receptors (FcεRI and FcεRII) on the surface of APCs, thereby facilitating the capture and internalization of antigen, which can subsequently be processed and presented to T cells (73). It has been demonstrated that IgE/FcεRI-mediated antigen uptake results in 100- to 1000-fold increased effectiveness of antigen presentation by APCs (74). A recent bronchial biopsy study has shown that pulmonary dendritic cells and mast cells stain positively for the α subunit of FcεRI, supporting the role of IgE-facilitated antigen presentation in the induction and maintenance of chronic inflammation in asthma (75).

Other cells, such as macrophages or B cells, may also be involved in antigen presentation, but their role in asthma is poorly understood. Increased FcεR1 receptor expression has been confirmed on bronchial tissue macrophages in atopic and nonatopic asthmatics, suggesting an involvement in IgE-mediated airway inflammation (50). Macrophages have the potential to promote inflammation through the production of pro-inflammatory mediators, such as PGE_2, hydrogen peroxide, leukotrienes, and cytokines (IL-1, TNF-α, IL-6, IL-8, IL-10, IL-12, GM-CSF, IFNγ, and TGF-β) (76), and it has been suggested that chronic activation of airway macrophages may be driving the inflammatory process in nonatopic asthma (77).

However, macrophages recovered by BAL are generally poor antigen-presenting cells and have predominantly an immunosuppressive role in the lungs (78,79). Alveolar macrophages (AMs) have a reduced capacity to bind with T cells, which may be due to reduced expression of LFA-1 on their cell surface (80), and defective expression of B7-1 (CD80) and B7-2 (CD86) previously shown on AMs would reduce the ability to present antigen efficiently to T cells due to reduced costimulatory signalling via CD28 and CTLA-4 (81). However, increased expression of CD86 (82) and CD80 (361) has recently been shown on AMs from atopic asthmatics compared with normal controls, suggesting that alveolar macrophages in asthma may be capable of antigen presentation.

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VII. Role of Costimulatory Molecules

There has been considerable interest in the role of costimulatory molecules in antigen presentation and T-cell activation and differentiation in asthma (Figs. 2 and 3) (83). The activation of T cells in immune responses requires at least two distinct signals from APCs, the first provided by the cognate interaction of the T-cell receptor (TCR) with MHC II–peptide complexes on APCs, and the second by the interaction of CD80 (B7-1) or CD86 (B7-2) on APCs with CD28 or CTLA on T cells (84). Costimulation via CD28 results in T-cell activation, while ligation of its higher affinity homolog, cytotoxic T-lymphocyte antigen (CTLA)-4, is considered to suppress the immune response by competing with CD28 for binding with CD80 or CD86 early in the immune response and preventing CD28-mediated activation (85). Lack of costimulatory signaling has been shown to result in T-cell unresponsiveness or anergy (86).

Murine studies suggest that CD28-B7 costimulation plays a critical role in the production of Th2 cytokines, the development of airway hyperresponsiveness,

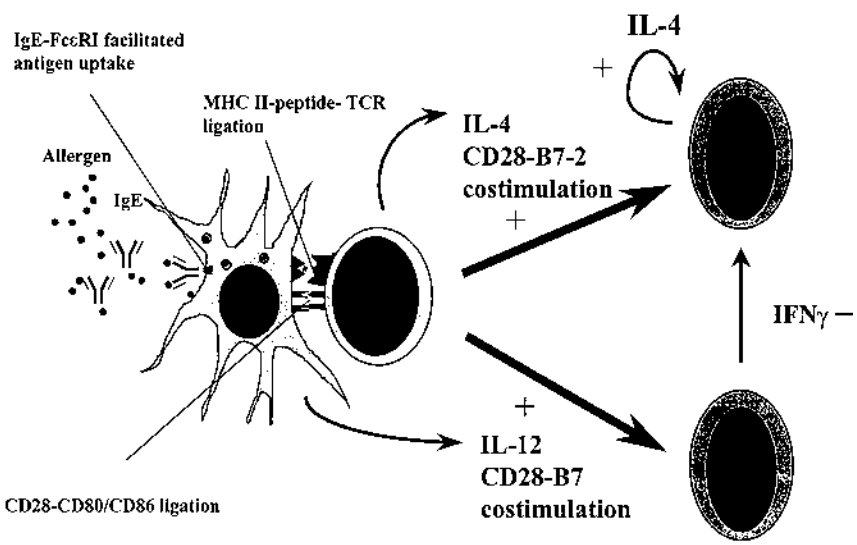

Figure 3 Role of costimulatory signaling via the CD28/CTLA4-CD80/CD86 pathway in the initiation and activation of the immune response, and the differentiation of naive Th0 lymphocytes towards a Th1 or Th2 phenotype. CD28-CD80/CD86 interaction in the presence of IL-4 promotes the development of a Th2-type immune response found in allergic inflammation, whereas costimulation in the presence of IL-12 or interferon gamma (IFNγ) deviates the immune response towards a Th1-type response and the development of cell-mediated immunity.

and eosinophilic airway infiltration in asthma (87,88). A recent study in mild asthmatics has shown a significant reduction of allergen-induced IL-5 and IL-13 production in bronchial tissue cultures by CTLA-4Ig, a humanized fusion protein of IgG_1 and the extracellular component of CTLA-4, that selectively antagonizes costimulatory signaling via the CD28-B7 pathway (89). CTLA-4Ig has also been reported to effectively inhibit allergen-induced synthesis of the chemokines IL-16 and RANTES in similar bronchial explant studies (90). The relevance of CD28-B7 costimulation in mild asthma has been further strengthened by Larche and colleagues, who have shown that a selective anti-CD86 antibody inhibits the allergen-stimulated proliferation of airway T cells isolated from BAL (91). The relevance of the CD28-B7 pathway in vivo and in more severe forms of asthma remains to be clarified and warrants further investigation. The application of CTLA-4Ig to clinical trials in asthma and other T-cell–mediated inflammatory conditions will be of particular interest (92).

VIII. The Bronchial Epithelium as a Regulator of Airway Inflammation

The bronchial epithelium is increasingly being shown to play a dynamic role in regulating the inflammation, repair, and remodeling process of asthma. It is well established as an important source of mediators including arachidonic acid products (15-HETE, PGE_2), nitric oxide (NO) endothelins, cytokines (IL-1β, IL-5, IL-6, IL-11, GM-CSF, IL-16, IL-18) (93–98), chemokines (IL-8, Gro-α, MCP-1, MCP-3, RANTES, MIP-1α, MIP-2, eotaxin, and eotaxin-2), and molecules involved in inflammation and repair.

Epithelium activation can occur via a number of stimuli including mediators such as histamine (99), cytokines (100–102), leukotrienes, and environmental stimuli including allergens, occupational chemicals or pollutants (103,104), viruses, and shifts in the epithelial lining fluid osmolarity that may occur in exercise-induced asthma. Inflammatory cells recruited to the airways release a number of cytokines, tissue-damaging proteases, and cationic proteins resulting in structural and functional damage to the epithelium. The mast cell–derived serine protease tryptase and Matrix-metalloproteinase-9 (MMP-9) derived from eosinophils stimulate IL-8 release and increased ICAM-1 expression by epithelial cells (105). Allergen-derived proteases (Der p 1, a cysteinyl protease, and Der p 6, a serine protease) have also been shown to promote IL-8 release and increase ICAM-1 expression by bronchial epithelial cells, possibly through protease-activated receptor (PAR) mechanisms (106). The protease activity of allergens such as Der p are also capable of epithelial cell detachment and cytokine production, most likely by interfering with the integrity of intercellular tight junctions on the epithelial barrier by cleavage of the tight junction adhesion protein oc-

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cludin (107). The disruption of the epithelial barrier by particular allergens would facilitate the delivery of antigens to APCs resident in the submucosa and accentuate the activation of the immune system (108,109). The confirmation of FcεRI and FcεRII receptors on bronchial epithelial cells (110) also suggests that epithelial cells can be directly activated by IgE-mediated mechanisms.

IX. Cytokine Networks in Asthma

It is increasingly evident that an expanding array of cytokines plays a critical role in orchestrating the airway inflammation in asthma by promoting the development, recruitment, activation, differentiation, and survival of inflammatory cells. Individual cytokines have many overlapping cell regulatory actions and function through complex cytokine networks (Fig. 2). They interact with specific high-affinity receptors on target cells, activating linked secondary intracellular cascades that regulate the specific transcription of genes and the ultimate cellular response. Considerable progress has been made in characterizing the cellular sources and actions of the numerous cytokines involved in asthma (Table 1).

Although in situ hybridization studies of BAL and bronchial biopsies have confirmed T cells to be a major source of cytokines in asthma, it is clear that other cells, including eosinophils, mast cells, and bronchial epithelial cells, and the structural elements of the airways contribute to the cytokine milieu. Cytokines derived from T cells and mast cells, such as IL-4, IL-5, and IL-13, play central roles in the pathogenesis of asthma by their ability to promote eosinophil recruitment, activation, terminal differentiation, and survival in the airways and, in the case of IL-4, promote B-cell switching to IgE production (11). IL-4 and IL-13 act in concert with TNF-α to stabilize vascular cell adhesion molecule (VCAM)-1 expression on endothelial cells, increasing the adherence of eosinophils to the endothelium, resulting in enhanced eosinophil recruitment to the airways (30,112). IL-3 and GM-CSF contribute to eosinophilic inflammation by increasing eosinophil survival, and mast cell and basophil development. A number of studies have confirmed increased expression of the Th2-type cytokines, which promote the allergic response (IL-4 and IL-5), and reduced expression of the Th1-type cytokine IFNγ, in BAL and bronchial biopsies from asthmatics (113,114). Although T cells and mast cells are likely to initiate the production of eosinophilotactic cytokines, eosinophils are also capable of enhancing eosinophilic inflammation by producing IL-4, IL-5, GM-CSF, and TNF-α (115). While there is a considerable body of evidence to support the role of cytokines such as IL-3, IL-4, IL-5, IL-6, and GM-CSF in asthma, a number of studies have recently focused on IL-10, IL-12, IL-13, and IL-16 (113,115–117).

There is evidence that IL-10 may participate in mitigating allergic inflammatory responses, although initial studies suggested predominantly pro-

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Table 1 Cellular Source and Actions of Cytokines Relevant to the Inflammatory Process in Asthma

Cytokine	Cell source	Actions
IL-1 (IL-1α, β & IL-1 Ra)	Monocytes, macrophages, smooth muscle, and endothelium	Activation of T cells and airway epithelial cells Promotes B-cell proliferation Initiates gene transcription
IL-2	T cells	Promotes T-cell proliferation and clonal expansion
	Eosinophils	Eosinophil chemotaxis
IL-3	T cells	Stimulates development of mast cells and basophils
	Mast cells	Promotes granulocyte differentiation and activation
	Eosinophils	Promotes eosinophil survival
IL-4	T cells	Promotes T-cell activation and differentiation to Th2 phenotype
	Eosinophils Mast cells	Promotes B-cell growth, differentiation, and class switching to IgE production
	Basophils	Promotes eosinophil recruitment by upregulation of VCAM-1 on vascular endothelial cells
IL-5	T cells, eosinophils, bronchial epithelium, mast cells	Promotes growth and differentiation of eosinophils and basophils Activates and prolongs survival of eosinophils
IL-6	Monocytes, macrophages, eosinophils, mast cells, and fibroblasts	Activation of hemopoietic stem cells Differentiation of T cells and B cells Costimulatory factor for immune cells
IL-8	Macrophages, eosinophils, T cells, mast cells, endothelial cells, fibroblasts, neutrophils, airway epithelial cells	Neutrophil activation and differentiation Chemotactic factor for primed eosinophils, basophils, and neutrophils
IL-9	T cells	Enhances mast cell growth

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IL-10	T cell	Inhibits T-cell proliferation and downregulates proinflammatory cytokine production
	B cells	Cofactor in mast cell growth and differentiation
	Macrophages	Chemotactic for CD8+ T cells
	Monocytes	
IL-12	T cells, monocytes, macrophages, dendritic cells	Inhibits Th2 development and cytokine expression
		Supresses IgE production
		Promotes Th1 phenotype and IFNγ production
IL-13	T cells, basophils, mast cells	Promotes B-cell differentiation, proliferation, and class switching to IgE production
		Promotes dendritic cell development
		Promotes eosinophil accumulation by increased expression of VCAM-1 on endothelial cells
IL-16	CD8+ T cells, mast cells, eosinophils, and airway epithelium	Activates monocytes and CD4+ T cells
		Recruitment of CD4+ T-cells eosinophils
IL-18	Macrophages airway epithelial cells	Activates B cells to produce IFNγ, promoting Th1 phenotype
GM-CSF	Macrophages, eosinophils, neutrophils, T cells, mast cells, airway epithelial cells	Priming of neutrophils and eosinophils
		Prolongs survival of eosinophils
TNFα	Mast cells, T cells, monocytes, neutrophils, epithelial cells	Enhances eosinophil and mast cell cytotoxicity
		Chemoattractant for neutrophils and monocytes
		Upregulates adhesion molecules
IFNγ	T cells, NK cells, macrophages, eosinophils	Suppression of Th2 T cells
		Inhibits B-cell differentiation
		Upregulates eosinophil activation markers CD69, HLA-DR, increases in vitro cytotoxicity and viability
		Increases ICAM-1 expression on endothelial and epithelial cells

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inflammatory effects of IL-10 in allergy by its ability to suppress Th-1 proliferation, differentiation and IFNγ production. Studies using in situ hybridization have shown reduced IL-10 expression in BAL from asthmatics compared to normal subjects, which is co-localized predominantly to T cells and alveolar macrophages (118). Interleukin-10 mRNA was increased after allergen challenge, coincident with the LAR, suggesting that IL-10 may act to dampen down inflammatory responses induced by the earlier release of pro-inflammatory cytokines (119). Interleukin-10 has been shown to reduce the expression of accessory molecules CD80, CD86, CD23, MHC II, on APCs, thereby suppressing accessory cell function and antigen presentation (120). IL-10 inhibits the release of the eosinophilopoeitic cytokine IL-5 by Th2 lymphocytes and accelerates eosinophil death by reducing expression of CD40 on eosinophils and inhibiting eosinophil-derived GM-CSF and TNF-α release (121). Interleukin-10 inhibits IgE-dependent production of TNF-α, GM-CSF, and IL-6 and has been shown to suppress allergen-stimulated proliferation and generation of IgE-positive B cells in PBMC cultures from atopic subjects (121). However, IL-10 is a growth factor for mast cells in rodents, and in vitro studies have shown that IL-10 increases IgE release by B cells, preactivated through CD40 in the presence of exogenous IL-4, suggesting that IL-10 may also exacerbate inflammatory responses. This suggests that IL-10 may be useful as a potential therapeutic agent in asthma by controlling Th2-mediated inflammatory processes and preventing eosinophil accumulation (115,119).

Recent interest has focused on the possible role of IL-12 in regulating airway inflammation in asthma. Interleukin-12 is derived predominantly from macrophages, monocytes, and dendritic cells, and a recent study by Naseer and coworkers has shown reduced mRNA expression for IL-12 in bronchial biopsies of atopic asthmatics compared to normal controls, which is localized predominantly to alveolar macrophages and T cells (113). The numbers of IL-12 $mRNA^+$ cells were increased significantly after treatment with prednisolone in steroid-sensitive (SS) asthmatics associated with improvements in lung function, but remained unchanged in asthmatics showing a poor clinical response to steroid treatment. IL-12 has been shown to suppress the development of IL-4–producing Th2 cells and to promote the development of Th1-type lymphocytes (22). In sensitized mice, IL-12 treatment has been shown to abrogate airway hyperresponsiveness, eosinophilia, and the production of Th-2-type cytokines after allergen inhalation (123). Interleukin-12 has also been shown to suppress IgE production by IL-4 stimulated PBMCs (124) and inhibit IL-5 release in allergen-stimulated PBMC cultures of allergic asthmatics (125). There has been considerable enthusiasm regarding the therapeutic potential of IL-12 replacement therapy in asthma (126). Administration of recombinant human IL-12 to mild asthmatics by weekly subcutaneous injection resulted in a significant reduction in circulating eosino-

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phils and a halving of eosinophil counts in induced sputum. However, there was no significant improvement in AHR or in EAR or LAR following inhaled allergen challenge (127).

The central role of IL-13 in chronic airway inflammation in asthma has recently been confirmed. These studies have shown overexpression of IL-13 in the lungs of transgenic mice results in an increase in airway mucus secretion, the development of subepithelial fibrosis, airway hyperresponsiveness, and eosinophilic airway inflammation. This was associated with increased pulmonary eotaxin expression, and the effects of IL-13 have been shown to be independent of IL-4 (128–130). This cytokine promotes the development and expression of MHC class II and FcεRII (low-affinity IgE receptor) molecules on dendritic cells (131,132) and also upregulates VCAM-1 expression on vascular endothelial cells (133), which together with IL-4 and TNFα promotes eosinophil accumulation. Interleukin-13 is produced mainly by activated T cells and has a potentially important role in regulating allergic inflammation in asthma by promoting B-cell proliferation, differentiation, and immunoglobulin secretion by enhancing isotype class switching from IgG to IgE. Studies have conclusively shown elevated numbers of cells expressing IL-13 mRNA in the bronchial tissue of atopic and nonatopic asthmatics (134) and increased IL-13 levels in BAL after segmental allergen challenge (131,135). As noted for IL-2 and IL-4, treatment with prednisolone results in improvement in lung function, which is associated with a significant reduction in IL-13 mRNA expression in glucocorticosteroid-sensitive asthmatics, but not in glucocorticosteroid-resistant asthmatics, who showed little clinical improvement after treatment (113).

IL-16 is a newly characterized cytokine that is produced by CD4+ and CD8+ T cells, epithelial cells, mast cells, and eosinophils (117,136,137). It uses the surface molecule CD4 as its ligand and thus activates cells that are CD4+, namely monocytes and CD4+ T cells (138). Bronchial biopsy studies using ISH have shown expression of IL-16 mRNA on epithelial cells from asthmatics but not normal or atopic controls. The epithelial and subepithelial IL-16 mRNA expression was significantly associated with airways hyperresponsiveness and CD4+ T-cell infiltration in the bronchial mucosa (94). IL-16 has been detected early in BAL postendobronchial allergen challenge in asthmatics but not in control subjects (117). This suggests a role for early IL-16 release in the selective recruitment of CD4+ T cells and eosinophils to the inflamed bronchial mucosa in asthma.

IL-18 is another recently characterized cytokine synthesized by activated macrophages and bronchial epithelial cells (139). In association with IL-12, IL-18 acts on Th1-type T cells to produce IFNγ, and in vivo and in vitro studies in mice show that IL-12 and IL-18 strongly activate B cells to produce IFNγ (140). Further studies are necessary to characterize the function of IL-18 in asthma.

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X. Transcription Factors

There is increasing awareness of the critical role played by transcription factors in the regulation of cytokine gene expression in asthma (141). Cytokines bind to specific receptors on target cells, resulting in increased intracellular production of an array of protein kinases and phosphorylation of transcription factors like nuclear factor kappa B (NE-κB) and activator protein-1 (AP-1) (142). These intracellular signaling proteins bind to regulatory sequences of target genes, resulting in the promotion (transactivation) or suppression (transrepression) of gene transcription and subsequent mRNA and protein synthesis.

NF-κB is thought to play a central role in allergic airway inflammation, but a number of other transcription factors, including the JAK-STAT pathway (Janus kinases-signal transducers and activators of transcription), C-EBPβ (C/enhancer-binding protein β, previously NF-IL-6), NF-AT (nuclear factor of activated t cells), AP-1, and GATA-3, also appear to be involved in Th-2 differentiation and allergic responses (143). In particular, elevated numbers of cells coexpressing GATA-3 mRNA and IL-5 mRNA in asthmatic airways have been shown to be CD3+ T cells, supporting the link between GATA-3 overexpression and IL-5 dysregulation in asthma (144). Many stimuli including cytokines (IL-1 β, TNF-α), viruses, and oxidants can stimulate the activation of NF-κB and AP-1 in the lung (145,146). NF-κB is an important regulator of several inducible genes, such as inducible nitric oxide synthase (iNOS) (147), the inducible form of cyclooxygenase (COX-2), pro-inflammatory cytokines, chemokines, and adhesion molecules (148).

Therapies for asthma, including corticosteroids and cyclosporin A, may function through interaction with transcription factors (149). Glucocorticosteroid receptors (GRs) are themselves transcription factors, and after binding with glucocorticoids in the cytoplasm, they localize to the nucleus and bind to positive glucocorticoid response elements (pGREs) or negative glucocorticoid response elements (nGREs) to promote or inhibit gene transcription (150). Corticosteroids also inhibit activation of other transcription factors like AP-1 or, in the case of NF-κB, via a direct interaction between the activated glucocorticosteroid receptor and the p65 subunit of NF-κB. In T cells and monocytes, glucocorticoids have also been shown to increase gene transcription for I-κBα, which binds to activated NF-κB in the nucleus and induces dissociation of NF-κB from κB-binding sites on target genes. In glucocorticoid-resistant asthmatics, there is exaggerated AP-1 production, which sequesters activated GRs, reducing their availability to inhibit the pro-inflammatory actions of NF-κB (151,152). Since it is likely that transcription factors such as NF-κB play a critical role in amplifying airway inflammation in asthma, an improved understanding of the functions of transcription factors may lead to the development of novel therapeutic agents.

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XI. Evidence of Airway Remodeling in Asthma

A number of studies indicate that irreversible airflow obstruction may occur in asthma, associated with structural changes consistent with an airway remodeling process (153). Morphometric studies have shown prominent smooth muscle hyperplasia and hypertrophy in both large and small airways (154), increased angiogenesis (155), prominent mucus gland hyperplasia (156), and airway wall thickening particularly in cases of fatal asthma (157).

The thickened basement membrane zone abnormalities noted at postmortem of asthmatics have been confirmed even in bronchial biopsies of mild asthmatics. Immunohistochemistry studies have shown that this thickened layer is composed of collagen types I, III, V, and fibronectin, most likely originating from myofibroblasts, and electron microscopy studies have localized the increased subbasement membrane thickness to the lamina reticularis layer (158,159). In vitro studies suggest that the remodeling process involves a number of growth factors and cytokines, including transforming growth factor (TGF)-β_1, platelet-derived growth factor, basic fibroblast growth factor, TNF-α, IL-4, endothelin, and other molecules including tryptase and histamine, thereby suggesting that mast cells contribute to airway remodeling in asthma (160,161) (Fig. 4). Activated eosinophils in the reticular lamina have been shown to be a major source of TGF-β_1 mRNA, which is closely associated with the degree of airway fibrosis and decline in FEV_1 seen in asthma, providing further links between chronic allergic airway inflammation and the structural remodeling process in asthma (162).

Tryptase is known to cleave certain components of the extracellular matrix (e.g., fibronectin, collagen VI) and activate MMPs (e.g., MMP-9, stromolysin) and can also enhance the proliferation of fibroblasts, smooth muscle, and epithelial cells (163). A number of extracellular matrix proteins contribute to the inflammation and remodeling of asthma. Eosinophils express integrins such as VLA-4, VLA-6, and LFA-1, which are known to adhere to matrix proteins such as laminin, fibronectin, and fibrinogen (164,165), which promote eosinophil accumulation, activation, and survival locally in the bronchial tissue (166). Mast cells also express integrins, and in vitro studies have shown that fibronectin adherence encourages IgE-mediated mast cell degranulation, chemotaxis, and proliferation. In asthmatics, matrix protein interaction is capable of altering IgE-mediated cytokine release by basophils (166).

Recent studies suggest that the epidermal growth factor receptor (EGFR) may play an important role in the repair of the epithelium in asthma (167). Overexpression of the EGFRs and C erb β1, but not C erb β2, β3, or β4, has been shown in both repairing and apparently normal epithelium in asthma (168,169), suggesting that the bronchial epithelium in asthma is chronically ''stressed'' and in a persistent repair phenotype. Epidermal growth factor receptor can be acti-

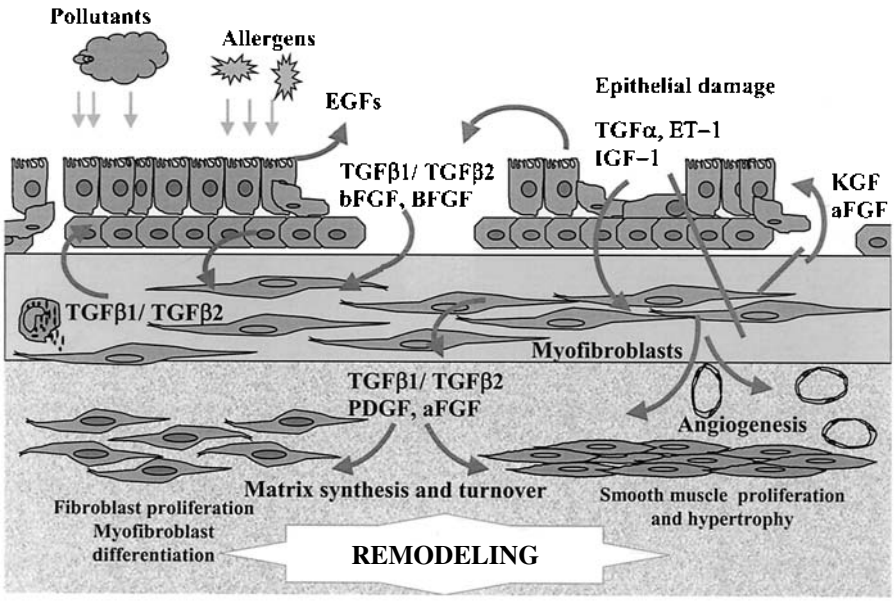

Figure 4 The epithelial mesenchymal trophic unit: a model of epithelial repair and remodeling in asthma. The bronchial epithelium and the underlying myofibroblasts release a number of cytokines and growth factors in response to injury, which promotes the proliferation of myofibroblasts and smooth muscle cells, angiogenesis, and the synthesis of tissue matrix, which plays an important role in the repair and remodeling process in asthma. The repair process has been compared with the interaction of the epithelium and mesenchymal tissue in the normal development of the human lung. EGF, epidermal growth factor; TGF-β1, transforming growth factor-β1; PDGF, platelet-derived growth factor; aFGF, activated fibroblast growth factor; ET-1, endothelin-1; KGF, keratinocyte growth factor.

vated by environmental stress, cytokines, growth factors, and G-protein–coupled receptors, supporting a role for EGFR in coordinating the response to mucosal injury and repair in asthma (167,170).

In contrast to epithelial wound healing elsewhere in response to damage, the bronchial mucosa reepithelialization occurs in the absence of granulation tissue formation. Similar to the epithelial-mesenchymal interactions involved in the branching morphogenesis in the embryonic lung, it has been postulated that a bidirectional communication occurs between the repairing epithelium and the underlying myofibroblasts in asthma resulting in a chronic imbalance of matrix synthesis and remodeling (Fig. 4) (167). The concept of an epithelial-mesenchymal trophic unit in asthma is supported by the identification that growth factors (KGF,

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EGF, basic and acidic fibroblast growth factors, and TGF-β itself) involved in normal lung development and airway branching (171,172) are also released by the repairing bronchial epithelium in response to injury (161,169,173). Keratinocyte growth factor (KGF), an epithelial mitogen, is also released by cultured myofibroblasts, which may also regulate the proliferation of basal epithelial cells, which have been shown to strongly express receptors for KGF.

A recent study involving bronchial biopsy in children as young as 3 years of age indicated that irreversible structural changes of airway remodeling are present at a very early stage in asthma (174) and that inhaled corticosteroids do not appear to significantly reverse the airway deposition of collagen despite relieving symptoms, improving lung function, and modifying bronchial hyperreactivity (175,176). This supports the current recommendation for the early introduction of effective anti-inflammatory treatment for all but the mildest forms of asthma (177).

XII. Intrinsic and Extrinsic Asthma: A Role for Local Airway IgE Production in Both?

The concept of allergic and nonallergic asthma has been challenged by epidemiological studies showing that the prevalence of asthma is closely related to the serum IgE concentration regardless of atopic status (4). At the molecular level, the underlying pathogenic mechanisms in both groups of asthmatics share a number of similarities but also exhibit certain distinct features. Intrinsic asthmatics have been classified as having serum IgE concentrations lying within the ''normal'' reference range (0–150 IU/mL), although total serum IgE concentrations were noted to be higher than nonatopic control subjects (50).

Both atopic and ''intrinsic'' forms of asthma exhibit airway infiltration by activated mast cells, eosinophils, CD4+ and CD8+ T lymphocytes, basophils, and neutrophils, but the level of circulating and airway eosinophilia is found to be more prominent in intrinsic asthmatics, as shown in bronchial biopsies and induced sputum (178). Intrinsic asthmatics display more pronounced activation of peripheral blood CD4+ and CD8+ T lymphocytes and CD45+ memory T cells (29,179). Ying and colleagues have identified elevated numbers of activated CD25+ T cells in bronchial biopsies of both atopic and intrinsic asthmatics (50,178), but the numbers of CD3+CD4+, but not CD8+ T cells were found to be slightly higher in intrinsic asthmatics (31).

A ''Th2 environment'' has been shown to be a strong component of atopic and nonatopic asthma, with increased expression of mRNA encoding the cytokines IL-3, IL-4, IL-5, IL-13, GM-CSF and augmented expression of the C-C chemokines, MCP-3, and RANTES (31,32). Bronchial mucosal IL-5 mRNA expression has been shown to correlate with markers of disease severity in both

atopic and nonatopic asthma (180). Elevated numbers of cells expressing mRNA for IL-4 have been shown in atopic asthma, which have been closely related with disease severity. The demonstration of increased numbers of cells expressing mRNA for IL-4 and IL-13 (134) and elevated numbers of cells bearing FcεRI receptors (50) in bronchial tissue of both atopic and nonatopic asthma suggests that local bronchial submucosal IgE production may contribute to the inflammatory process in intrinsic asthma. The putative role of local airway IgE synthesis in intrinsic asthma has been supported by the documented increased expression of ε germ-line gene transcripts (Iε) and mRNA for the ε heavy chain of IgE in bronchial biopsies from both atopic and nonatopic asthmatics. Thus, in atopic and nonatopic asthma there may be a significant role for IgE (77), possibly directed against a yet unidentified allergen in intrinsic asthma.

It has also been suggested that intrinsic asthma may be a consequence of persistent macrophage activation as a result of an auto-allergic process triggered by a low-grade viral infection. Abnormal macrophage activation in intrinsic asthma has been described (181), and increased numbers of FcεRI-bearing macrophages have been identified in asthmatic airways, suggesting a role for IgE-mediated antigen presentation (50). Bentley and colleagues have shown markedly increased numbers of CD68+ macrophages in bronchial biopsies from intrinsic asthmatics, which exhibited increased expression of the activation marker GM-CSF-α receptor subunit (178). Increased numbers of cells expressing mRNA for IL-3 and GM-CSF are also found in the bronchial mucosa of intrinsic asthmatics (182). IgE-mediated production of chemokines (IL-8, MCP-1) and pro-inflammatory (TNF-α, IL-1, IL-6) and anti-inflammatory (IL-1 receptor antagonist, IL-10) cytokines has been demonstrated in human alveolar macrophages mediated by IgE-CD23 interaction (183). It has been suggested that airway inflammation is preferentially promoted by the activation of IgE receptors on macrophages in nonatopic asthma.

XIII. IgE Synthesis in the Asthmatic Airway

In order to produce a functional IgE molecule, B cells must undergo two DNA rearrangement events, a $V_H(D)$ J_H recombination, which gives rise to an IgM-IgD- B cell, and a rearrangement involving isotype switching, which gives rise to a B cell expressing IgG, IgA, or IgE. A two-signal hypothesis has been postulated for the induction of IgE synthesis (184).

A. Induction of IgE Synthesis: A Two-Signal Model

The first signal is supplied by the activity of cytokines, IL-4/IL-13; these molecules are responsible for determining isotype specificity, i.e., immunoglobulin gene transcription at a specific region of the Ig locus in B cells (185,186). The

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second signal can be generated by multiple mechanisms, which results in the activation of DNA switch recombination. These signals include contact-mediated signals delivered by T cells (184), B-cell activators including Epstein-Barr virus (EBV) infection (187,188), monoclonal antibodies to the B-cell antigen CD40 (188), and hydrocortisone (189,190). The signals required for IgE production are generated following the interaction of activated T cells with B cells. Activation of T cells by APCs such as dendritic cells, macrophages, or B cells via the MHC II-TCR and CD28-B7 costimulatory pathways leads to the secretion of cytokines including IL-4 and/or IL-13 and the expression of CD40 ligand (CD40L) by the T cell. Alternatively, it was considered that a noncognate interaction might be involved, where the class II MHC antigen/peptide complex is not recognized by the T-cell receptor (191). Both of these mechanisms could activate the CD4+ T cells that are required to induce IgE synthesis (192,193) (Fig. 5).

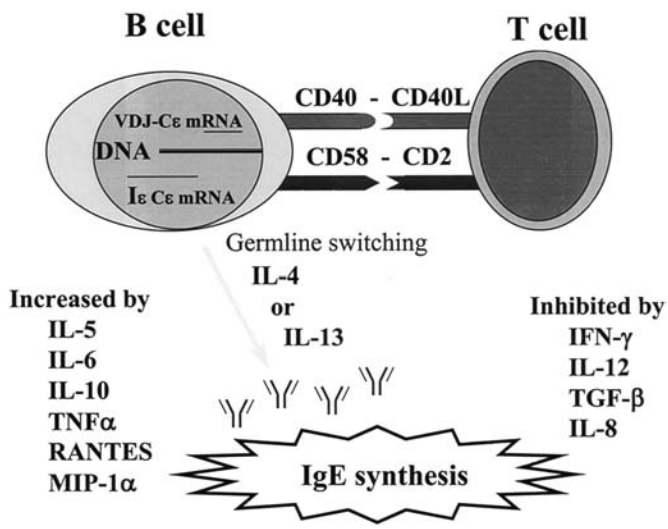

Figure 5 T- and B-cell interactions involved in the induction of IgE synthesis. Allergen binds to antigen-specific IgM on the surface of B cells, which leads to the internalization of the IgM/Ag complex followed by endosomal processing and the presentation of antigen-derived peptides via the HLA class II molecule. The T-cell receptor (TCR) complex specifically recognizes this HLA class II/Ag complex, leading to the induction of CD40L expression. CD40L interacts with CD40 on the surface of the B cell, stimulating an increase in the expression of CD80 surface antigens; the CD80-CD28 interaction is a costimulatory signal that leads to the induction of cytokine expression and T-cell proliferation. The cytokines IL-4/IL-13 bind to receptor complexes on B cells and in the presence of the CD40 signal results in B-cell proliferation, IgE isotype switching, and synthesis.

B. Interleukin-4 and Interleukin-13

Substantial evidence has supported the role of IL-4 and IL-13 in the induction of IgE synthesis (194). Interleukin-4 and IL-13 are the only cytokines known that can induce IgE synthesis in vitro using the recombinant proteins (186,195). The role of IL-4 was initially identified in vitro, T-cell–derived IL-4 was able to induce IgE production by lipopolysaccharide (LPS)-stimulated murine B cells (196), and anti-IL-4/IL-4 receptor (IL-4R) antibodies inhibited IgE responses in experimental animals (197,198). The role of IL-4 in human IgE isotype switch in B cells from IgM to IgE has been established using normal unfractionated peripheral blood mononuclear cells (PBMCs) (199) and single B cells stimulated with murine and human T-cell clones (192,200). Interleukin-4 is produced by activated Th2-type CD4+ cells and Tc2 type CD8+ cells (201), mast cells, and basophils (202). Functions attributable to IL-4 include upregulation of surface CD23, MHC class II and IL-4 receptor expression on monocytes and B cells, and increased expression of VCAM-1 on endothelial cells.

There is compelling evidence for the role of IL-13 in the induction of IgE synthesis (132,186,203). Interleukin-13 is produced by activated Th2-type CD4+ and Tc2-type CD8+ T cells, basophils, and mast cells (204–206) and shows similar biological effects to IL-4, including upregulation of CD23 and MHC class II expression on B cells and monocytes and induction of ε germline transcription and isotype switching to IgE/IgG_4 (207,208). Although IL-13 and IL-4 share several functions, only IL-4 can act as a growth factor for T cells (209). The regulation of IL-13 and IL-4 production is also thought to be regulated by independent mechanisms. T cells stimulated with anti-CD28 and phorbol myristate acetate (PMA) results in optimal production of IL-13 with almost no production of IL-4 (210). IL-4 activity is regulated on several levels and is thought to involve a naturally occurring splice variant of human IL-4, IL-4 delta 2, which can act as an antagonist for IL-4–induced IgE synthesis and CD23 upregulation in B cells (211).

The signal delivered by IL-4 or IL-13 initiates transcription through Iε, the switch region Sε, and the Cε exons. This spliced 1.8 Kb mRNA germline transcript is truncated and nonfunctional due to the presence of stop codons in all reading frames of Iε. IL-4/IL-13 mediate their functions via the interaction with cell-bound and soluble from receptors (212). IL-4R is composed of two subunits, a 145 kDA IL-4Rα chain required for IL-4 binding and the signal transduction of growth-promoting and transcription-activating functions and a common γc chain found in several cytokine receptors, which amplifies signaling of the IL-4R α chain (Fig. 6)(213). IL-4R is expressed on T cells, B cells, mast cells, macrophages and the endothelium (214). IL-13R has a heterodimeric structure and is composed of IL-13Rα involved in IL-13 binding and the IL-4Rα chain (215).

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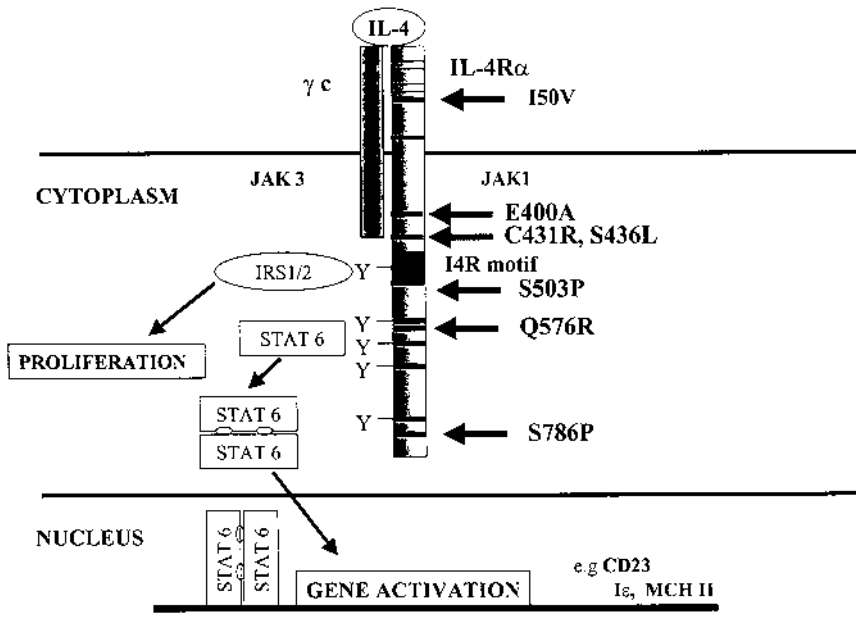

Figure 6 IL-4 receptor α chain polymorphisms. The IL-4 receptor is composed of an α chain (IL-4Rα or αa′) and a γ chain (γ_c), but other forms do exist due to the formation of heterogeneous structures with components of the IL-13 receptor. Signal transduction via IL-4R is thought to involve the activation of IRS1/2 and STAT6, which leads to cell proliferation and the activation of gene transcription at several loci in the genome including several cytokine genes, CD23 and Iε. The positions of known polymorphic sites within the coding region of the gene are also highlighted. A role for the 150V variant in determining the activation level of the receptor has been suggested by Mitsuyasu et al. (225), but this is still controversial due to the polymorphic nature of the receptor and the concept of which polymorphism is actually resulting in the functional effects.

Therefore IL-4 and IL-13 can engage the IL-13R due to the presence of both cytokine specific α chains, which has obvious functional implications (Fig. 7). The signal transduction pathways involved in IL-4R–mediated activities have been elucidated; IL-4Rα and γc chains are associated with the Janus family tyrosine kinases, namely JAK1 and JAK3 (216). Upon receptor activation, these kinases phosphorylate tyrosine residues in the intracellular domains of the receptor, which then serve as binding sites for signal transducer and activator of transcription (STAT) molecules, namely STAT6 (217). Subsequent phosphorylation of STAT6 molecules generates active homodimers, which translocate to the nucleus

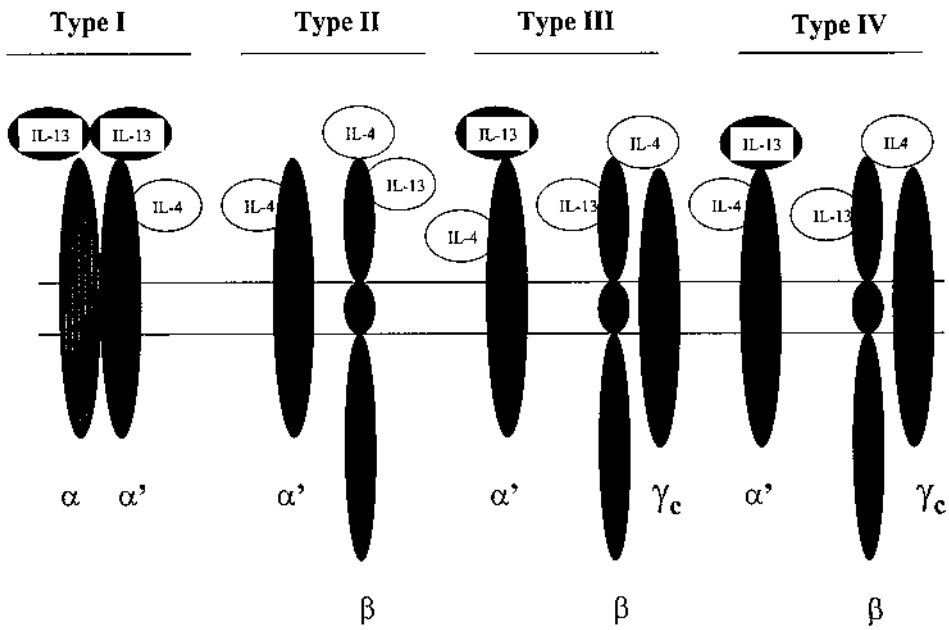

Figure 7 Models for IL-13 receptor structures. The diagram represents the proposed composition of the four IL-13 receptor complexes described by Obiri et al. (360). Type I IL-13R is composed of two 65–70 kDa isomers, α and α', respectively, which both bind IL-13. The α' chain also binds IL-4, but in this configuration IL-13 will have greater affinity for the receptor than IL-4. Type II IL-13R may be composed of the α' subunit and a 140 kDa IL-4Rβ subunit, which is shared between IL-4R and IL-13R. This receptor binds both ligands, but IL-13 binding to IL-4Rβ is low affinity in contrast to IL-4 binding to the α' subunit. This receptor therefore results in cross-competition by IL-13 and IL-4 for each others' binding region. Type III is predicted to be composed of the α', IL-4Rβ, and a 64 kDa subunit known as the common γ (γ_c) chain due to the finding that this subunit is present in multiple cytokine receptor structures. In this arrangement IL-13 will bind weekly to IL-4Rβ and will interact with the α' subunit. The γ_c subunit does not engage IL-13, but as shown IL-4 binds to both the IL-4Rβ and γ_c subunit. Again IL-13 and IL-4 will compete for binding for this receptor. Type IV may be composed of the α' subunit, which binds both IL-4 and IL-13, the IL-4Rβ subunit, which binds IL-4 and IL-13, but IL-13 with very low affinity, and the γ_c subunit, which interacts with the IL-13R complex and binds IL-4. Therefore, IL-4 will bind to all three proteins, which explains the observation that IL-4 binds well to cells expressing this complex and IL-13 demonstrates only a limited binding. (Adapted from Ref. 360.)

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and in the presence of other transcription factors, such as NF-κB, can initiate transcription of multiple genes including Cε (204) (Fig. 6). This pivotal role of STAT6 has been reinforced by the observation that STAT6 knockout mice lack IgE class switching (218). Signal transduction via the IL-13R is thought to involve STAT6 activation leading to Cε transcription, possibly using a JAK2 (219) and TYK2 (220) mechanism. An independent signal transduction pathway related to IL-4–mediated cell growth activities has also been described (202,204,221–223), possibly involving IL-4–dependent phosphorylation of insulin receptor substrate 1 (IRS-1), SH2 domain–containing molecules, phosphatidylinositol 3 kinase (PI-3 kinase), and mitogen-activated kinase (MAPK) molecules.

Interestingly, the IL-4Rα gene has been found to be highly polymorphic (Fig. 6), containing 12 identified single nucleotide polymorphisms, 5 of them leading to amino acid substitutions: Ileu50Val, Glu375Ala, Cys406Arg, Ser411Leu, and Ser761Pro (224). Characterization of these variants has led to controversy due to the presence of multiple mutations confounding the analysis. Residue 50 of the IL-4R α chain is located in the extracellular portion of the receptor; an Ileu50Val variant has previously been described (213). It was shown that the Ileu 50 variant was associated with atopic asthma but not with nonatopic asthma in a Japanese population and also showed association with raised total serum IgE, house dust mite–specific IgE, and atopy in children (225). In vitro transfection studies using mouse and human B-cell lines have demonstrated that the Ileu 50 transfected mouse cells showed almost threefold greater cell growth and an approximately threefold increase in luciferase activity (expression under the control of the Iε promoter) compared to the Val 50 transfected cell line in response to human IL-4 (225). These IL-4R polymorphisms require further characterization to evaluate linkage disequilibrium effects and demonstrate that the regulation of IgE synthesis in individuals may have a genetic predisposition leading to high or low secretion levels (Fig. 6).

Although it is considered that IL-4 or IL-13 stimulation is necessary but not sufficient for the induction of IgE synthesis in B cells, with a particularly dominant role played by IL-13 (226), an additional ''second'' signal delivered by CD40-CD40L interaction is also required.

C. CD40 and CD40L

The role of CD40/CD40L in IgE synthesis has been of particular interest. Early work illustrated that recombinant IL-4 and anti CD40 antibodies could induce the synthesis of high levels of IgE by purified B cells in culture (188). It was found that an existing high level of CD40 expression on B cells was upregulated in atopic individuals, and anti-CD40 antibody alone was found to cause the synthesis of IgE. In nonatopic individuals, only antibody and recombinant IL-4 could promote IgE synthesis. CD40 has been identified as a 39 kDa surface phosphory-

lated glycoprotein expressed on primary mature B cells, follicular dendritic cells (FDCs), and normal endothelium. It has been demonstrated that activated γ/δ T cells expressing CD40L can induce isotype switching in B cells (227). CD40 activation by CD40L leads to B-cell adhesion, increased CD80/86, CD23, ICAM-1, and lymphotoxin-α (LT-α) expression (228). CD40-mediated triggering of the isotype switch is postulated to be under the control of a unique signal transduction pathway, involving a TNF receptor–associated factor (TRAF)–mediated process (229–231) or protein tyrosine kinase (PKC)–mediated pathways (232,233). However, due to the lack of a kinase domain in CD40, it is suggested that CD40-associated proteins must be involved in signaling, possibly via the JAK kinase pathway (194).

In addition to the stimulatory signals described above, the low-affinity IgE receptor, FcεRII/CD23, is thought to have a prominent role in the regulation of the IgE response. CD23 can be cleaved into soluble fragments of variable length (sCD23); these fragments have been suggested to have cytokine-like activities (234). Various roles for the membrane-bound CD23 molecule have been found; the B-cell CD23 molecule has been shown to present allergen in the form of an allergen-IgE-CD23 complex to T cells; it has also been suggested that CD23 expression is required as a ''third'' component in the induction of IgE producing cells and for these cells to become memory cells (235).

IL-4/IL-13 upregulates CD23 expression on B cells and a range of inflammatory cells providing a local source of contact stimulation and soluble growth factor. IgE itself can exert a negative feedback effect by binding to the membrane form of CD23. The mechanism of action is thought to involve the cell adhesion molecule CD21, which is a counter receptor for CD23. CD23 can bind and trigger CD21 on B cells and enhance IgE synthesis (236). Therefore a potential role for CD21 may be clonal amplification of B cells by antigen. IgE could exert its effects by binding to the same or overlapping site(s) on the CD23 molecule as CD21; it also is thought that the binding of IgE to CD23 prevents the release of the soluble forms of the receptor (237,238). The CD23-CD21 interaction has been further investigated, confirming that antibodies to CD23 can inhibit IL-4–induced human IgE production and by the identification of the site on the CD21 molecule that interacts with CD23 (239). More recently it has been shown that a soluble form of the CD21 receptor (sCD21, 135 Kda) is generated in vivo, which inhibits sCD23 induced IgE synthesis in IL-4–stimulated B cells (240). The role of sCD23 in the induction of IgE synthesis has also been demonstrated by the finding that inhibition of proteolytic processing of CD23 inhibits IgE synthesis (241).

D. The Role of Other Cytokines in IgE Synthesis

In addition to the prominent role of IL-4, it has been postulated that IL-5 (B-cell growth factor) and IL-6 (B-cell differentiation factor) may have a role in the

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regulation of the IgE response in asthma (199,242,243). In the presence of IL-4 both cytokines increase IgE synthesis in PBMCs, which is inhibited by the addition of anti-IL-6. The role of IL-6 in the induction of IgE synthesis following and IL-4/CD40 stimulation has been reinforced more recently (244,245). IFNγ, IFNα, and TGF-β have all been shown to suppress IL-4–induced IgE synthesis (198,242,246,247). IL-4 has been shown to downregulate IFNγ production (248), and IFNγ has been found to be an antagonist for IL-4–dependent induction of CD23 on B cells. IL-12 also suppresses the production of mature ε transcript while having no effect on ε germline transcription (124). In a mouse model of asthma, elevated IL-9 has been shown to produce higher serum IgE levels, possibly by a modulation of the IL-4 mechanism (249).

XIV. The Role of FcεRI and CD23 in Allergic Disorders

A. The High-Affinity IgE Receptor

The high-affinity receptor (FcεRI) engages IgE with a 1000 higher affinity ($k_A \sim 10^{10}\ M^{-1}$) compared to FcεRII/CD23, which binds IgE with relatively low affinity ($k_A \sim 10^7\ M^{-1}$). FcεRI is expressed on a variety of cell types, initially found on mast cells and basophils, and more recently identified on the surface of epidermal Langerhans cells (250), eosinophils, (251), peripheral blood monocytes (252), blood dendritic cells (253), epithelium cells (254), and human platelets (255). FcεRI has been implicated in several functions, including IgE-mediated hypersensitivity, antigen presentation (256), and IgE-dependent killing of schistosomes (251). FcεRI is considered a critical component of the effector arm of the allergic response in mediating both the immediate and late allergic response; indeed elevated numbers of cells expressing FcεRI have been described in mild asthma patients compared to controls (50). The extensive distribution of both Fcε receptors on a variety of cell types found in the asthmatic lung has confounded the identification of which IgE receptor is mediating which function. Clearly, FcεRI on mast cells and basophils has a defined role in the initial triggering of the allergic cascade, and IgE-FcεRI complexes on dendritic cells (257) and macrophages (50) have a role in enhanced allergen presentation to T cells leading to T-cell–mediated allergic reactions. The role of FcεRI on other cell populations has been the focus of some conflicting reports in the literature. FcεRI is found on eosinophils, the primary effector cell in the asthmatic lung, and it has been reported that FcεRI upregulation on BAL eosinophils is a key component in the asthmatic response (46). Recently it has been suggested that IgE-FcεRI interaction does not activate eosinophils and an IgG-IL-5 mechanism was postulated (258). Evidence also exists for a role of IgE-mediated activation or regulation of eosinophils via the FcεRI receptor (259). These findings highlight the need for further research into the precise role of FcεRI on these cell types and in the pathogenesis of allergic disorders.

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B. FcεRI Structure-Function Correlations

The FcεRI receptor is composed of four transmembrane polypeptides, which are noncovalently attached comprising the FcεRIα chain (232aa), FcεRIβ chain (244aa), and two disulfide-linked FcεRIγ chains (68aa) (260). The α chain is a member of the Ig superfamily, and its extracellular (EC) domain (45–65 kDA) contains the IgE-binding site; the β (32 kDa) and γ chains (7–9 kDa) are required for surface expression of the receptor complex and transmembrane signaling. The FcεRIγ cytoplasmic tail contains an immunoreceptor tyrosine activation motif (ITAM), and γ phosphorylation has been linked to signal transduction. This has been confirmed using chimeric constructs (261).

The receptor composition is $\alpha\beta\gamma_2$ (262), but hFcεRI has also been found as $\alpha\gamma_2$ complex (263,264). It has been demonstrated that the presence of the β subunit is essential for full IgE-mediated activation of mast cells (265). Previously, models based on homology to known structures have been used as tem-

Figure 8 (Panel 1) Structural models of human IgE-Fc in the coplanar (A) and bent conformation (B). The location of the regions implicated in the interaction of hIgE with its receptors are shown in the context of structural models of IgE in the coplanar and bent conformation. The predicted structures of hIgE-Fc are based on sequence homology with IgG_1, the demonstration of the parallel nature of the inter-Cε2 disulfide bonds, and biophysical data, which suggest that IgE bends at the Cε 2/3 and/or Cε 3/4 interface (266,270,279–282). Colors in the original art correspond to: Cε2 (green), Cε3 (yellow), and Cε4 (violet). Sequences thought to influence IgE/FcεRI binding include the Cε2-3 interface (aa330-335) (black) (274,275,283,284), the A-B loop, aa343-353 (red) (278,285), aa306–313, 311–320, 331–338, 382–391 (orange) (286), and residues R376, S378, K380, D414, R427, M429 (blue) (287). Regions that have been implicated in FcεRII/CD23 binding include aa367-376 (cyan) (276), the Cε2-3 interface (black) (286), and the A-B loop (278). (Panel 2) The high-affinity Fc receptor α chain (FcεRIα). (A) The hFcεRI α-chain extracellular domains (domain 1 a.a 1-86, domain 2 a.a 87-172) were modeled using homology to with the constant domain of a murine κ -light chain (267). The predicted domain structure of seven anti-parallel β strands, arranged as two β sheets (three and four strands) linked with a single disulfide bond is clearly visible, the seven sites of N-glycosylation, which do not contribute to the complementary interaction, have been omitted. (B) This illustrates regions of domain 2 thought to be involved in the interaction with IgE including: a.a 100-115 (288), a.a 93-104, 111-125, 123-137 (289), a.a 87-128, 129-145, 146-169 (290), a.a 119-129 (291), and D159, K117 (292). (Panel 3) The low-affinity Fc receptor (CD23/FcεRII). (A) Schematic representation of the hFcεRII/CD23 molecule depicting the triple helical coiled coil stalk region that has been computed to connect the extracellular lectin homology domains with the N-terminal cytoplasmic domain. The single N-glycosylation site is also shown. (B) Model of lectin-homology domain (268). Regions involved in IgE binding are colored in the original; residues 176–183 (red), 185–190 (violet), 224–233 (green), 238–243 (yellow), and 251–256 (cyan) (293). (Adapted from Ref. 272.)

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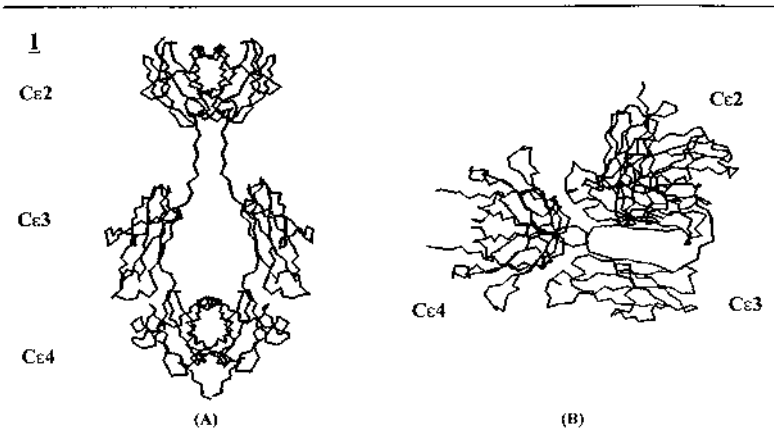

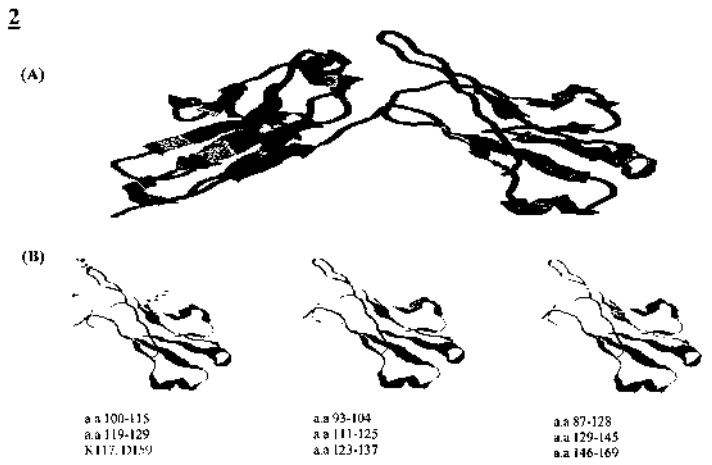

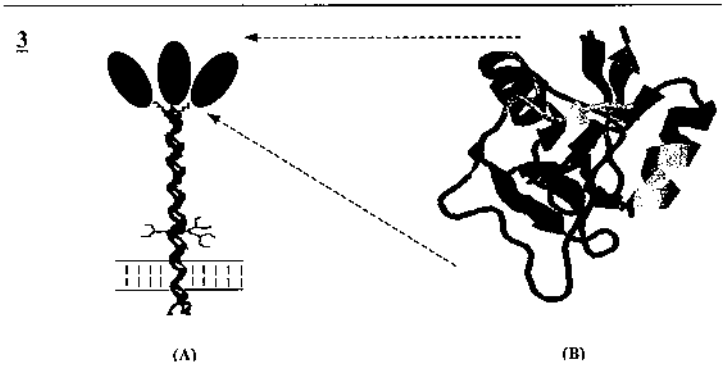

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plates for structure-function studies of the IgE-FcεRIα interaction (266–270). More recently the structures of the two FcεRIα extracellular Ig domains have been crystallized, leading to new insights into the nature of the IgE-FcεRI interaction (271).

C. The Human IgE-FcεRI Interaction

The main binding site(s) on the IgE molecule are located within the Cε3 domain and domain 2 of FcεRIα (272) (Fig. 8). The k_A of this interaction has been shown to be $\sim 10^{10}\ M^{-1}$ in numerous studies using cell-based assays and using surface plasmon resonance (SPR) in conjunction with a recombinant soluble form of the FcεRIα extracellular domains applying a simple pseudo first-order model (273). SPR measurements indicated that the IgE/FcεRI interaction is biphasic (274,275), which has been confirmed in a later study following an empirical analysis (273). The precise mode of interaction, however, requires further investigation. Glycosylation of either molecule is not required for the interaction (260,276,277). A pH dependency has been demonstrated for the interaction of hIgE with FcεRI, with optimum receptor occupancy at 6.4 and 7.4, suggesting a role for His residues in hIgE in the maintenance of the high-affinity of interaction (278).

The interaction is thought to involve a 1:1 binding stoichiometry, even though the IgE molecule contains two ε chains and has therefore two potential binding sites. It has been rationalized by the finding that IgE in solution, when receptor engaged, is bent from the planar position (280) (Fig.8). The most obvious explanation for the 1:1 stoichiometry would be that the bent conformation renders the second ε chain inaccessible via steric effects involving the Cε2 domain twisting over the face of Cε3. One explanation is that conformational changes occur during binding and/or that the binding of the α-chain to one site may prevent the interaction with the second site. Interestingly, the removal of structural constraints associated with the mutation of Pro333 to A or G at the Cε2-3 interface of IgE dramatically reduces the ability of IgE to mediate stimulus secretion coupling, although the effect on the kinetics of interaction is minimal. This suggests that this residue may play a role in the formation of a bent IgE and that a decrease in structural constraints computed to accompany the mutation of Pro to Gly inhibits the ability of the ligand to mediate receptor aggregation and secretory responses (273). Further studies to identify the molecular basis of this unexpected observation are necessary since they hold promise for the design of FcεRI-blocking agents, which cannot induce mediator secretion upon crosslinking (272,273).

D. The Low-Affinity IgE Receptor (FcεRII/CD23)

CD23 has been implicated in a number of functions including B-cell growth and differentiation and IgE-mediated immunity. It was independently characterized as the B-cell receptor for IgE but has now been found on eosinophils, platelets,

natural killer cells, T cells, follicular dendritic cells, Langerhans cells, and epithelial cells (294). The role of CD23 is still not fully understood, although it appears that the function of the molecule is tissue specific. Two forms of CD23 exist (FcεRIIa and FcεRIIb), which differ by only a few residues in the N-terminal portion of the protein. It has now been shown that these forms are functionally distinct; FcεRIIa mediates endocytosis by B cells and FcεRIIb is involved in IgE-mediated phagocytosis by a diverse number of cells including monocytes and eosinophils (295,296). Interestingly, a recent publication has shown expression of both forms of CD23 on the surface of eosinophils (297).

CD23 binds IgE in a species-specific manner (monomeric form k_A 10^7 M^{-1}, dimers and complexes k_A 10^8 M^{-1}) and the expression of the receptor is modulated by IgE possibly preventing proteolysis of the extracellular region (298). Autoproteolysis of CD23 produces receptor fragments including 37, 33, 29, 25, and 16 kDa fragments, and these soluble forms of the receptor have been shown to have diverse cytokine-like properties, originally being described as IgE-binding factors (IgE-BF), although they can function independently of IgE. It has been shown that expression of CD23 and IgE-BFs is under the control of IL-4 and IFNγ, depending on the cell line under study. IL-4 generally has an upregulation effect, with obvious implications in allergy, where it was found that expression of CD23 and IgE-BFs was required for an IgE response (299,300). A second ligand for CD23 has also been identified—CD21—which is involved in the modulation of the IgE response (236,301). CD21 is a membrane protein found on B cells, follicular dendritic cells, and basophils and has recently been identified in a soluble form (240).

The CD23 molecule has been shown to be involved in combating parasitic infestation (296); the induction of IgE synthesis (302,303); the maintenance and modulation of the IgE response (237,239,304); B-cell proliferation, activation, and differentiation (238,305); and as a cell adhesion molecule (301). The findings that CD23 interacts with β integrins, CD11b and CD11c, on monocytes (306) and that CD21 is expressed on the surface of several cell types (239) indicate that the role of CD23 is far beyond a low-affinity IgE receptor. The IgE-Cd23 interaction is considered to have pivotal role in both the immediate and LAR of asthma. This is exemplified by the finding that CD23 interactions mediate IgE production, that IgE-CD23–dependent activation of alveolar macrophages leads to the production of chemokines and cytokines, which promote airway inflammation (183), and that enhanced IgE-CD23 allergen presentation to T cells has been shown to be critical for pulmonary eosinophilia and Th2 cytokine production (307).

E. FcεRII Structure-Function Correlations

CD23 is a type II integral membrane glycoprotein with an apparent molecular weight of 45–55 kDa and is not a member of the Ig superfamily. The protein

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contains 321 residues with no N-terminal signal sequence and is composed of two regions separated by a 24-amino-acid trans-membrane region near the N terminal (308) (Fig. 8). The receptor is thought to be orientated so that the N terminal is found on the cytoplasmic side, and therefore, the composition is a large C-terminal extracellular region, a single TM, and an N-terminal cytoplasmic tail. The extracellular region contains a C-type (calcium-dependent) lectin domain, which places the receptor in a family of proteins that includes the asialogycoprotein receptor (ASGPR) and adhesion proteins known as selectins. This lectin domain is flanked on the C-terminal side by a sequence of unknown tertiary structure that contains an inverse RGD (Arg-Gly-Asp) sequence, implying a role in cell adhesion (305). On the N-terminal side of the lectin domain there is a repetitive region composed of five heptadic repeats of hydrophobic Leu/Ileu residues, and it is predicted that these repeats form an α helical stalk which is involved in trimer formation (309,310) (Fig. 8). It has been shown that two forms of human CD23 exist that differ structurally at the N terminal, where the seven terminal amino acids of FcεRIIa (NH_2-Met-Glu-Glu-Gly-Gln-Tyr-Ser) are replaced by six amino acids (NH_2-Met-Asn-Pro-Pro-Ser-Gln) in IL4-inducible FcεRIIb (295,296). These different forms are produced by alternate transcriptional initiation and RNA-splicing sites and have been shown to be tissue specific and functionally distinct.

No high-resolution structure exists for human CD23/FcεRII, which has led to modeling studies on the basis of homology to rat mannose-binding protein (rMBP) (267–269).

F. The Human IgE-FcεRII–CD23 Interaction

In contrast to the IgE-FcεRI interaction, the IgE-FcεRII–CD23 complex is thought to involve a 2:1 binding stoichiometry, with two lectin heads interacting with the two ε chains of an individual IgE molecule. It is believed that there are two binding sites on IgE that interact with residues on the dimeric/trimeric FcεRII (311). Residues within the Cε3 A-B loop and a region close to the glycosylation site at N371 play a critical role in the interaction of IgE with the lectin head region of the receptor (273,278) (Fig. 8). The IgE-binding site in FcεRII-CD23 has been identified—residues 151–288 in the lectin head region (311). Homolog scanning mutagenesis has been used to define the IgE-binding region on FcεRII further, implicating segments comprising residues 165–190 and 224–256 (293). There is a distinct region on the molecule to which all the critical segments identified by Bettler and coworkers (293) contribute, while the opposite face is probably involved in the interaction with glycosyl residues. This face of the molecule has been predicted to be analogous to the carbohydrate-binding region of rat MBP and is implicated in the interaction between FcεRII-CD23 and CD21-CR2, which is Ca^{2+} dependent and inhibited by fucose-1-phosphate (293). In contrast,

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there is no evidence for a contribution of carbohydrate to the complementary interaction between IgE and FcεRII. In fact, enzymatically deglycosylated IgE (276) or IgE variants where the site of carbohydrate attachment has been mutated engage FcεRII-CD23 with 10-fold increased binding affinity (I. Sayers et al., unpublished observations).

XV. Early and Late Asthmatic Responses: Role of IgE

A. IgE-Mediated Hypersensitivity: Basic Mechanisms

The role of IgE antibodies in the initiation of the allergic cascade is well established (312,313) and is characterized by a biphasic response involving an ''immediate or early allergic response'' (EAR) and a ''late phase allergic response'' (LAR) (314) (Fig. 9).

IgE plays an important role in the pathogenesis of asthma by virtue of its capacity to bind specifically to its two Fcε receptors. Mast cells sensitized with IgE via FcεRI interact with soluble multivalent allergen leading to receptor aggregation. In a series of unidentified steps, including the recruitment of intracellular protein kinases, phospholipases, and Ca^{2+} mobilization, a rapid degranulation occurs resulting in the release of preformed and newly synthesized pro-inflammatory mediators. These include, e.g. histamine, tryptase, leukotrienes, prostaglandins, platelet-activating factor (PAF), and a number of cytokines including IL-3, -4, -5, -6, -10, and -13, tumor necrosis factor (TNF)-α and GM-CSF (205). The immediate response occurs within minutes and in asthma is characterized by the constriction of the bronchioles and bronchi, contraction of smooth muscle, and vasodilation of capillaries. Lipid mediators produced include prostaglandins, PAF, and the leukotrienes, which have a prolonged effect and may therefore influence the allergen-induced LAR, which occurs 4–24 hours after initial challenge. This LAR is characterized by cell-mediated eosinophilic inflammation in the airway. Twenty-four to 48 hours after allergen challenge, infiltrating T cells express primarily mRNA for IL-4, IL-5, and GM-CSF but not IFNγ (316). In asthma the intensity of the LAR dictates the severity of bronchial hyperresponsiveness that follows allergen provocation (317). There is considerable debate as to whether this LAR component involves IgE-mediated release of mediators (318) or a form of cell-mediated hypersensitivity or both. Langerhans cells express FcεRI and have demonstrated efficient allergen presentation to T cells via an IgE-mediated process (319), suggesting that IgE has a multifunctional role in the allergic response that goes beyond the initial triggering of the allergic cascade (320). The LAR is considered to reflect the changes that occur after chronic allergen exposure, i.e., a strong inflammatory component with activated CD4+ T cells, tissue eosinophilia, and an increase in the production of the Th2-type cytokines (316).

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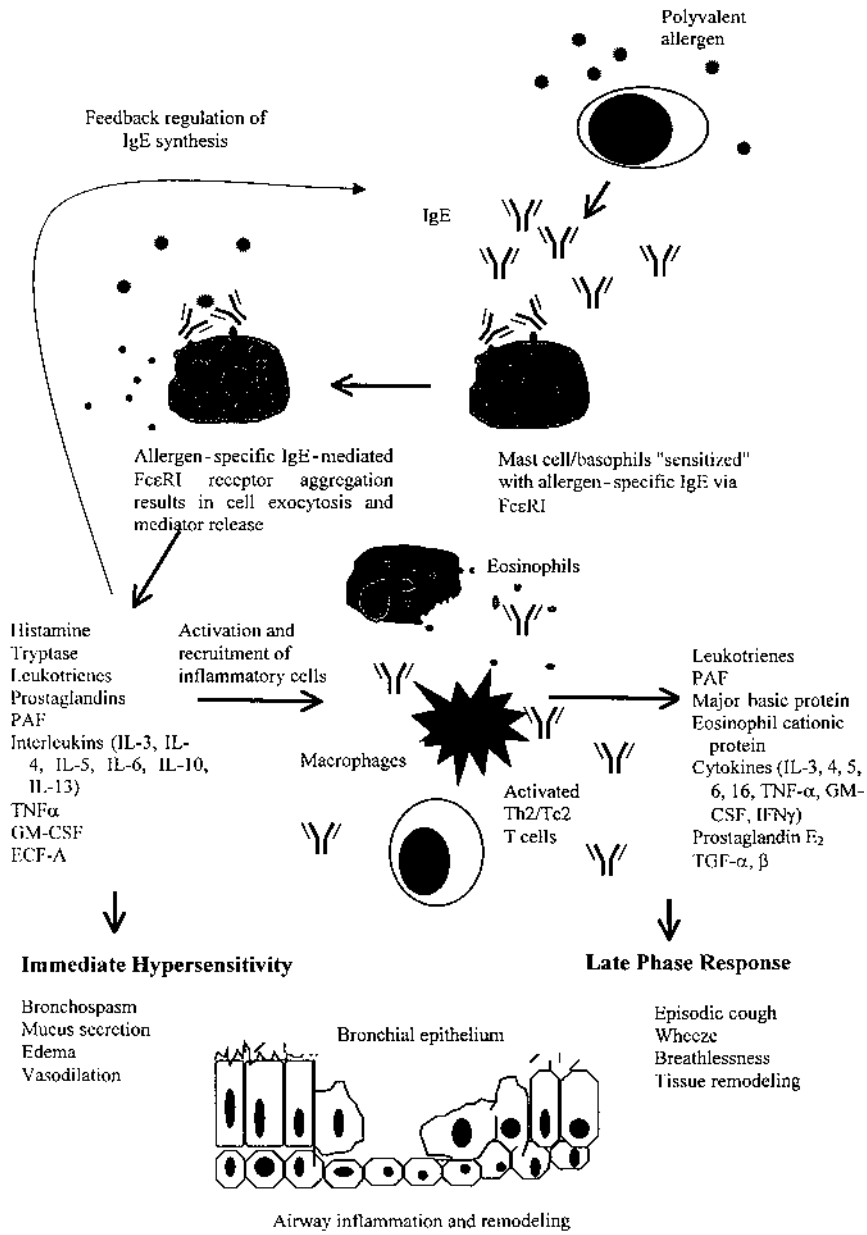

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Bronchial biopsies in stable atopic asthmatics have observed these components (19,64). Several Th2-type cytokines, (IL-4, IL-5, IL-13, but not IFNγ) and C-C chemokines are produced by multiple cells types, which regulate IgE synthesis and lead to the generation of eosinophilic airway inflammation (Fig. 9) (321). Allergic tissue damage results from the release of basic proteins, leukotrienes, and PAF secreted by activated eosinophils. It is clear that the mast cell plays an important role in the immediate response and that T cells play a key role in orchestrating the nature and severity of the inflammation by the secretion of cytokines. As stated earlier, the eosinophil is regarded as the primary effector cell due to the capacity to secrete major basic protein and eosinophil cationic protein, which have profound cytotoxic effects on the airway epithelium (316).

B. Critical Role of IgE in Immediate and Late Phase Responses of Allergic Asthma: Evidence from Clinical Trials Using Anti-IgE

IgE antibodies are crucial mediators of allergic reactions; the capacity of IgE to bind to its Fc receptors represents a potential site for therapeutic intervention. Of particular interest is inhibition of the IgE-FcεRI interaction due to the importance of FcεRI-mediated exocytosis in the initiation of the allergic cascade (48). The IgE-CD23 interaction is also a rationale target due to the role of the role CD23-IgE–allergen complexes in enhanced antigen presentation (72). These concepts have led to extensive studies in order to elucidate the structural basis of IgE-Fc receptor interactions as a prelude to the design of structural antagonists, which could prevent the initiation of the allergic cascade (272,282). The existence of naturally occurring anti-IgE antibodies is well established, mainly of the IgG isotype and having the ability to enhance or inhibit IgE-FcεRI binding (322,323). These findings led to the rationale that a nonanaphylactogenic anti-IgE antibody could block the IgE-FcεRI interaction and attenuate the allergic cascade.

For human therapy a nonanaphylactogenic monoclonal murine IgG1 anti-IgE (Mae11) was generated that specifically bound to circulating human IgE and recognized the identical or overlapping binding epitope as the α chain of the

Figure 9 IgE-mediated hypersensitivity in asthma. Following IgE synthesis and secretion from B cells, IgE rapidly binds to high- and low-affinity receptors on multiple cell types (sensitization), including mast cells and basophils via FcεRI. Crosslinking of receptor-bound IgE on mast cells by polyvalent allergen leads to receptor aggregation and cell activation, resulting in the secretion of preformed and newly synthesized mediators, which cause the clinical manifestations of immediate or type I hypersensitivity. These mediators also contribute to the late phase response through the priming and recruitment of inflammatory cells, which bear and upregulate CD23. Together the immediate and late phase responses lead to the clinical symptoms of asthma.

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FcεRI receptor (FcεRIα) and CD23 lectin head region in the Cε3 domain of hIgE. The humanized rhuMAb-E25 anti-IgE antibody has been shown to cause a dose-dependent decrease in serum IgE (324) and has been used in human clinical trials, which has led to a greater understanding of the role of IgE in the early and late phase responses in asthma.

In a randomized, double-blind, parallel group study of mild allergic asthmatics, allergen broncho-provocation in the presence and absence of intravenous administered rhuMAb-E25 was performed (325). The concentration of allergen causing a 15% decrease in FEV_1 was measured (PC_{15}), compared to baseline values, and the PC_{15} on days 27, 55, and 77 were increased by 2.3, 2.2, and 2.7 doubling doses, respectively, with rhuMAb-E25 and -0.3, $+0.1$, and -0.8 doubling doses with placebo ($p = 0.002$). Methacholine challenge PC_{20} also improved marginally, becoming significant on day 76 ($p < 0.05$). This inhibitory effect was associated with an 89% reduction in circulating IgE levels, suggesting that IgE has a pivotal role in the immediate response and that anti-IgE therapy may form a new novel treatment for asthma. A further randomized, double-blind, placebo-controlled study was performed by Fahy et al. (326). The administration of an intravenous infusion of RhuMAb-25 to mild atopic asthmatics reduced serum IgE levels, increased the allergen dose required to provoke an early asthmatic response, and decreased the mean maximal fall in FEV_1 during the early response (30 $\pm$ 10% at baseline to 18.8 $\pm$ 8% (treatment) vs. 33 $\pm$ 8% at baseline to 34 $\pm$ 4% after placebo; $p = 0.01$) and reduced the mean maximal fall in FEV_1 during the late phase response (24 $\pm$ 20% at baseline to 9 $\pm$ 10% vs. 20 $\pm$ 17% at baseline to 18 $\pm$ 17% after placebo; $p = 0.047$). Maximal broncho-constriction during the late phase was reduced by 60%, methacholine PC_{20} was increased by 1.6 doubling doses, and the degree of eosinophilia in induced sputum 24 hours after allergen challenge was reduced 11-fold. These findings provide compelling evidence for the role of IgE in regulating airway narrowing, airway tissue eosinophilia, and airway hyperreactivity in the LAR (326). Of interest, daily administration of rhuMAb-E25 directly to the airways for 8 weeks failed to inhibit early and late responses to allergen challenge, indicating that removal of circulating IgE was critical for efficiency (327).

Identification of the IgE-specific receptor/effector cell mechanism in the LPR is still outstanding. Enhanced IgE-CD23 allergen presentation to T cells has been shown to be critical for pulmonary eosinophilia and Th2 cytokine production in a murine model (307). In addition a role for IgE-FcεRI–mediated allergen presentation by dendritic cells to T cells has been shown to be critical for T-cell–mediated allergic reactions (257), thus providing rationale explanations for the role of IgE in the LAR. It is unlikely that the interaction of IgE with FcεRI on eosinophils plays a significant role in the LAR due to the finding that eosinophil activation results from IgG stimulation and the activity of cytokines, such as IL-5, independent of IgE-mediated activation (258). It has been suggested that CD4+ T

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cells control the inflammatory airway in the LAR via the release of Th2-type cytokines (328,329). Therefore, one possible explanation for the role of IgE in the LAR is in the regulation of T-cell activation via allergen presentation. Conflicting evidence has suggested a role for IgE-mediated in the activation and regulation of eosinophils via the FcεRI receptor (259).

The application of anti-IgE antibodies in animal studies and its use in clinical trials in mild forms of asthma has established the involvement of IgE in the pathogenesis of both the early and late allergen responses in asthma. The precise role of IgE and its high- and low-affinity receptors in the modulation of the LAR is still outstanding at this time, although increasing evidence suggests a role in allergen presentation and the activation of T cells as a primary function.

XVI. Molecular Therapeutic Strategies for Asthma

Corticosteroid treatment has been shown to be extremely effective in the management of the majority of asthmatic subjects (330), presumably by preventing the transcription of genes involved in airway inflammation (331), inhibition of cytokine (34,332) and chemokine release (333), suppression of inflammatory cell activity (334), and reducing the expression of adhesion molecules involved in inflammatory cell recruitment (335). However, significant numbers of asthmatics develop considerable morbidity related to side effects from the corticosteroids required for disease control, and a minority of asthmatics remain truly resistant to the immunomodulatory effects of current anti-inflammatory treatments such as corticosteroids (36,336) and the leukotriene receptor antagonists (337). This has prompted the development of more selective anti-inflammatory agents for the management of airway inflammation in asthma. The increased understanding of the complex cellular and molecular basis of asthma inflammation has led to the identification of key players that regulate the immunological cascade of asthma inflammation, which may be amenable to modulation by selective T-cell antagonists; immune deviation, cytokine supplementation or antagonism; inhibition of adhesion molecule expression; chemokine receptor blockade; or the administration of nonanaphylactogenic humanized anti-IgE molecules (Fig. 10).

A. Antagonists of IgE-Fcε Receptor Interactions

The inhibition of IgE-Fc receptor interactions would be highly desirable; of particular interest is the IgE-FcεRI interaction due to the importance of FcεRI-mediated exocytosis in the initiation of the allergic cascade (48). Approaches have focused on structural intervention of these interactions, including the use of novel recombinant proteins or peptides based on the sequence of IgE or FcεRI (282) or using monoclonal antibodies against human IgE (338), FcεRII (339), and FcεRI (340). The identification of binding regions on IgE and FcεRIα has led

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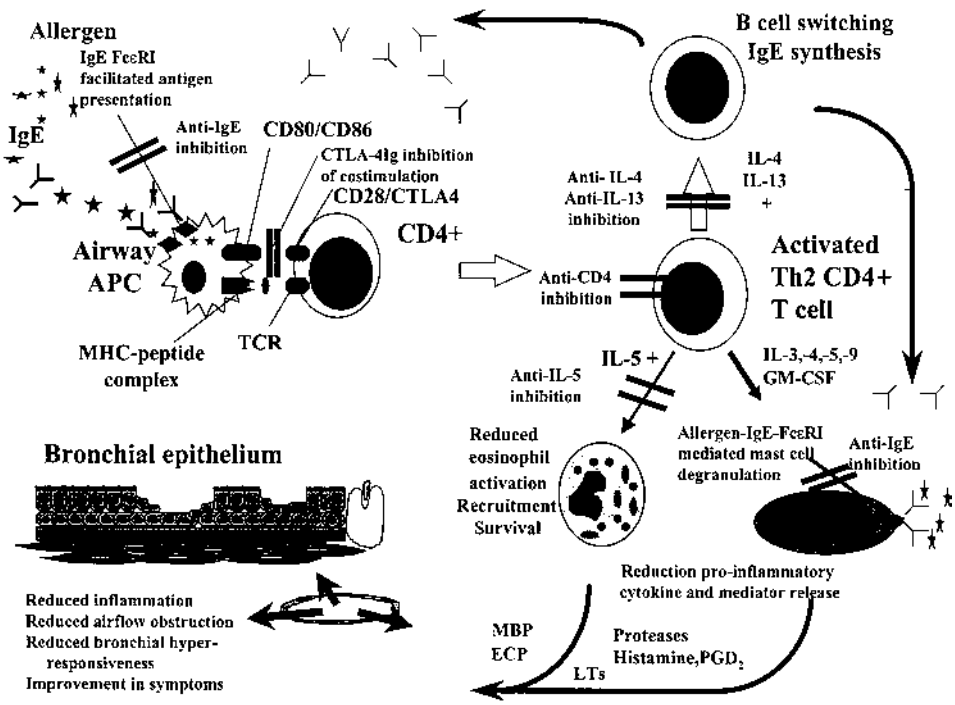

Figure 10 Effects of selective therapeutic antagonists on the inflammatory cascade in asthma.

to the design of constrained peptides, and it has been shown that the binding of IgE to the α chain of FcεRI can be blocked by both IgE- and receptor-derived peptides. The therapeutic utility of these peptides is, however, doubtful in view of the short half-life and 10^4- to 10^7-fold lower affinities compared with the native molecules. Furthermore, the administration of receptor-derived peptides carries the inherent danger of eliciting an antipeptide immune response, which can activate mast cell degranulation through receptor cross-linking. A phenomenon observed recently may also assist the development of more effective and safer antiallergic drugs. It emerged that when Pro333 at the Cε2-3 interface of hIgE was substituted by Gly, only a small alteration in the binding kinetics of the FcεRI interaction was evident, although FcεRI-mediated stimulus secretion coupling was completely abrogated (273). It is tempting to speculate that an understanding of this phenomenon may assist the design of inhibitors that can actively compete with hIgE for receptor sites while inhibiting FcεRI-mediated function.

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Investigations leading to the development of the humanized nonanaphylactogenic mouse monoclonal (mAb) antihuman IgE antibodies appear particularly promising, since these antibodies are potent inhibitors of the IgE-FcεRI interaction. The humanized rhuMAb-E25 monoclonal anti-hIgE antibody resulted in a dose-dependent reduction in serum free IgE levels leading to attenuation of both the early and late phase responses to allergen provocation, and it was well tolerated (341).

These intervention strategies can be considered as passive immunotherapy; it may, however, be possible to actively immunize atopic individuals since there is evidence that neonatal or adult immunization with IgE can stimulate the formation of therapeutically useful anti-IgE auto-antibodies in animal systems (342). Their action suggests that the identification of structural determinants in IgE, which participate directly in IgE-receptor interaction, may form the basis for the development of peptide immunogens to induce the formation of antibodies, which can bind specifically to the receptor-binding sites of IgE in the circulation and to membrane IgE on B cells. Used as vaccines, such peptides could have a direct application in the treatment of all IgE-mediated allergies.

B. Inhibition of IgE Synthesis: Modulation of the IL-4/IL-13 Receptor System

As described previously, the IL-4/IL-13 receptor system is critical in the activation of IgE synthesis; several approaches have been explored to selectively inhibit these functions. A mutant form of human IL-4 has been engineered—IL-4Y124D—containing a tyrosine-to-aspartate substitution at position 124. This variant engages IL-4R but lacks the T-cell proliferative response associated with IL-4 and inhibits IL-13 activity and IL-4/IL-13–induced IgE synthesis in vitro (343–345). Administration to *scid* mice reconstituted with B and T cells resulted in significant reduction of IgE production in the absence of any effect on IgG concentrations (346) Human IL-4 has also been shown to exist as an alternative splice variant whereby exon 2 is removed from the mature protein, IL-4δ, and has IL-4 antagonistic properties (347). Similarly, a splice variant of the IL-4R has been identified in mice which generates a soluble form of the receptor (212). Both of these splice variants occur in vivo and therefore constitute a naturally occurring control mechanism for IL-4 activity. All of these findings demonstrate that functional antagonists of the IL-4/IL-13 receptor system hold promise in the therapy of allergic disorders. However, due to the heterogeneity of the IL-13 receptor and a role for both IL-4 and IL-13 in the induction of IgE synthesis, the elimination of both cytokine activities is required to constitute a rationale therapy (Fig. 10). Interestingly, an IL-13–binding protein has been identified in mice, which binds IL-13 with greater affinity than IL-13R, suggesting a regulatory role

(348). Although IL-4 and IL-13 regulation provide compelling targets for therapy, once the IgE isotype switch has occurred, IgE synthesis is no longer dependent on IL-4 and therefore the elimination of IgE-secreting cells is required in parallel to IL-4/IL-13 modulation.

C. T-Cell Antagonists in Asthma

Recent studies using the T-cell–selective agent cyclosporin A (CsA) have shown promising results in the management of steroid-dependent asthma. Trials of CsA treatment in steroid-dependent asthma have resulted in improved lung function, a reduction in asthma exacerbations, and a reduction of IL-2 and IL-2 receptor expression and the transcription and translation of mRNA for IL-5 and GM-CSF in CD4+ T cells (349).

A recent trial investigating the effects of keliximab, a chimeric primatized® selective anti-CD4 monoclonal antibody in corticosteroid-dependent asthma, also supports the role of CD4+ T cells in chronic asthma. Administration of a single intravenous infusion of keliximab at doses ranging from 0.5 to 3.0 mg per kilogram body weight resulted in a significant reduction in circulating CD4+ T-cell counts. Even at 24 hours, significant improvements in lung function and symptom scores were documented that persisted for 14 days in patients receiving the higher dose of 3.0 mg/kg (350). Although still an experimental treatment, this may represent a useful adjunctive treatment in the management of severe asthma.

Selective antagonism of CD28-mediated T-cell costimulation with CTLA-4Ig has been shown to be well tolerated and effectively suppress T-cell–mediated parameters of disease severity in patients with psoriasis (351). As an increasing number of reports are documenting the importance of T-cell costimulation in the pathogenesis of asthma, this supports the initiation of clinical trials assessing antagonists of T-cell costimulation, such as CTLA-4Ig, in the management of asthma (92).

D. Anti-IL-5 Antibodies

The selective yet central role played by IL-5 in regulating the development of eosinophils in bone marrow and the accumulation, activation, and survival of eosinophils in the airways in asthma has identified the potential of antagonists as a novel therapeutic strategy in asthma. Studies using a blocking monoclonal anti-IL-5 antibody in animal models of asthma including primates have shown significant improvements in airway hyperresponsiveness and eosinophilic airway inflammation (352). Phase 1 clinical trials of a humanized anti-IL-5 antibody (SK-240563) have recently been performed, and it has been shown to effectively reduce eosinophil counts in the circulation and induced sputum of mild asthmatics. Of particular therapeutic interest was the observation that the reduction in eosinophil counts was noted to persist for 3 months after a single I.V. injection

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(353). Although significant improvements in airway hyperresponsiveness or response to allergen challenge were not achieved, the prolonged activity of the agent on eosinophil counts is promising and merits assessment of anti-IL-5 therapy in more severe forms of asthma.

E. Anti-TNF-α

Elevated TNF-α expression has been documented in asthmatics (354), particularly after allergen challenge, and in acute severe asthma (355) and is considered to play an important role in airway inflammation and bronchial responsiveness in asthma (356). TNF-α is predominantly released by mast cells by IgE-dependent mechanisms and is known to augment the release of pro-inflammatory mediators and chemokines and the expression of adhesion molecules, leading to inflammatory cell recruitment and activation in the airways in asthma. Recent clinical trials of a blocking humanized anti-TNF-α monoclonal antibody have shown dramatic clinical benefit in the management of acute exacerbations of rheumatoid arthritis. The pro-inflammatory activity in asthma supports the potential of TNF-α antagonism in suppressing airway inflammation in asthma.

F. Interleukin-10 Replacement

The recognition of a relative deficiency of IL-10 in asthma and its potential anti-inflammatory activity by inhibition of cytokine release has prompted interest in the potential of IL-10 replacement therapy in asthma. Recombinant human IL-10 has been administered as a weekly infusion to patients with inflammatory bowel disease, where a similar pattern of cytokines is expressed, and has been shown to be remarkably effective in disease control (357).

G. Interleukin-12

There has been considerable enthusiasm regarding the potential of IL-12 replacement therapy to reset the dysfunctional Th2 immune response in asthma (126). Recombinant human IL-12 has been administered to humans and has been shown to be safe in low doses. Systemic or topical administration of IL-12 may prove valuable in the treatment of more severe forms of asthma.

H. *Mycobacterium vaccae*

There is interest in the potent immuno-modulating effects of extracts derived from *Mycobacterium vaccae*, a *Mycobacterium* species that normally occurs as an environmental saprophyte. It is currently being investigated as an immunotherapeutic agent for tuberculosis, HIV, cancer, and allergic asthma and has been well tolerated. Administration of this agent to patients with tuberculosis has been shown to restore a protective Th1-type immune response (358). Pretreatment of

BALB/c mice with an extract from *M. vaccae* has been shown to suppress the allergic response to ovalbumin challenge, resulting in reduced IgE and IL-5 release, which supports the therapeutic potential for allergic asthma. Clinical trials involving *M. vaccae* as an immunotherapy in asthma are currently underway.

XVII. Conclusion

The exponential increase in the understanding of the complex immune pathways in asthma inflammation has led to the development and clinical trials of agents capable of modulating the activity of inflammatory cells in asthma. There is now a considerable body of evidence that IgE-mediated mechanisms contribute significantly to the complex immunological pathways involved in the activation of inflammatory cells, the production of cytokines, and the release of pro-inflammatory mediators that effect the epithelial damage, repair, and remodeling process so characteristic of asthma. Increased knowledge of the structural composition of high- and low-affinity IgE receptors, the airway cells that express these receptors, and the functional activity of IgE-FcεRI and IgE-FcεRII interactions has led to the development of novel molecular therapies that suppress IgE-mediated inflammation, such as the anti-IgE antibody RhuMAb-E25. Future efforts will have to be directed at elucidating the relative contribution of individual cell types and mediators in the hope that the wide syndrome of asthma may eventually be broken down into subtypes, hopefully with distinct patterns of mediators that are involved in their pathogenesis. Of particular importance is the development of selective antagonists capable of abrogating the inflammatory response in more severe disease, which is not only becoming more prevalent, but is also draining a major proportion of the funds dedicated to asthma management (359).

References

1. ISAAC. Worldwide variation in prevalence of symptoms of asthma, allergic conjunctivitis, and atopic eczema: ISAAC. The International study of Asthma and Allergies in Childhood (ISAAC) steering committee. Lancet 1998; 351:1225–1232.
2. Sears M. Epidemiology. Asthma: Basic Mechanisms and Clinical Management. 3rd ed. Chicester, England: Academic Press Ltd., 1998:1–33.
3. Burrows B, Sears M, Flannery E, Herbison G, Holdaway M. Relationship of bronchial responsiveness assessed by methacholine to serum IgE, lung function, symptoms, and diagnoses in 11-year old New Zealand children. J Allergy Clin Immunol 1992; 90:376–385.
4. Burrows B, Martinez F, Halonen M, Barbee R, Cline M. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989; 320:271–277.

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5. Kelly W, Hudson I, Phelan P, Pain M, Olinsky A. Atopy in subjects with asthma followed to the age of 28 years. J Allergy Clin Immunol 1990; 85:548–557.
6. Roorda R, Gerritsen J, van Aalderen W, et al. Risk factors for the persistence of respiratory symptoms in childhood asthma. Am Rev Respir Dis 1993; 148:1490–1495.
7. Peat J, Tovey E, Toelle B, et al. A major risk factor for childhood asthma in Australia. Am J Respir Crit Care Med 1996; 153:141–146.
8. Sporik R, Holgate S, Platts-Mills T, Cogswell J. Exposure to house dust mite allergen (Der p 1) and the development of asthma in childhood. A prospective study. N Engl J Med 1990; 323:502–507.
9. Frischer T, Meinert R, Karmaus W, Urbanek R, Kuehr K. Relationship between atopy and frequent bronchial response to exercise in school children. Paediatr Pulmonol 1994; 17:320–325.
10. Sunyer J, Anto J, Sabria J, et al. Relationship between serum IgE and airway hyperresponsiveness in adults with asthma. J Allergy Clin Immunol 1995; 95:699–706.
11. Sears M, Burrows B, Flannery E, Herbison G, Hewitt C, Holdaway M. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. N Engl J Med 1991; 325:1051–1067.
12. Platts-Mills T. Dust-mite allergens and asthma—report of a second international workshop. Clin Immunol 1992; 89:1048–1060.
13. Lau S, Falkenhorst G, Weber A, et al. High mite-allergen exposure increases the risk of sensitisation in atopic children and young adults. J Allergy Clin Immunol 1989; 84:718–725.
14. Vervloet D, Charpin D, Haddi E, et al. Medication requirements and house dust-mite exposure in mite-sensitive asthmatics. Allergy 1991; 46:554–558.
15. O'Hollaren M, Yunginger J, Offord K. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. N Engl J Med 1991; 324:359–363.
16. Pollart S, Chapman M, Fiocco G, Rose G, Platts-Mills T. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989; 83:875–882.
17. Celenza A, Fothergill J, Kupek E, Shaw R. Thunderstorm associated asthma: a detailed analysis of environmental factors. B Med J 1996; 312:604–607.
18. Salvaggio J, Aukrust L. Mould-induced asthma. J Allergy Clin Immunol 1981; 68: 327–346.
19. Azzawi M, Bradley B, Jeffery PK, et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthmatics. Am Rev Respir Dis 1990; 142:1407–1413.
20. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 1992; 326:298–304.
21. Bousquet J, Chanez P, Lacoste JY, et al. Eosinophilic inflammation in asthma. N Engl J Med 1990; 323:1033–1039.
22. Holgate ST. Asthma: a dynamic disease of inflammation and repair. Ciba Found Symp 1997; 206:5–28.
23. Sur S, Crotty TB, Kephart GM, et al. Sudden-onset fatal asthma—a distinct entity

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

with few eosinophils and relatively more neutrophils in the airway submucosa. Am Rev Respir Dis 1993; 148:713–719.
24. Koshino T, Arai Y, Miyamoto Y, et al. Airway basophil and mast cell density in patients with bronchial asthma and relationship to bronchial hyperresponsiveness. J Asthma 1996; 33:89–95.
25. Robinson DS, Bentley AM, Hartnell A, Kay AB, Durham SR. Activated memory T helper cells in bronchoalveolar lavage fluid from patients with atopic asthma: relation to asthma symptoms, lung function, and bronchial responsiveness. Thorax 1993; 48:26–32.
26. Corrigan C, Hartnell A, Kay AB. T lymphocyte activation in acute severe asthma. Lancet 1988; 1(8595):1129–1132.
27. Hamid Q, Barkans J, Robinson D, et al. Co-expression of CD25 and CD3 in atopic allergy and asthma. Immunology 1992; 75:659–663.
28. Hamid Q, Azzawi M, Ying S, et al. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. J Clin Invest 1991; 87:1541–1546.
29. Walker C, Bode E, Boer L, Hansel T, Blaser K, Virchow KJ. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine productionin peripheral blood and broncho-alveolar lavage. Am Rev Respir Dis 1992; 146:109–115.
30. Ying S, Durham SR, Corrigan CJ, Hamid Q, Kay AB. Phenotype of cells expressing mRNA for TH2-type (interleukin 4 and interleukin 5) and TH1-type (interleukin 2 and interferon gamma) cytokines in bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects. Am J Respir Cell Mol Biol 1995; 12:477–487.
31. Ying S, Humbert M, Barkans J, Corrigan CJ. Expression of IL-4 and IL-5 mRNA and protein product by CD4+ and CD8+ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and non-atopic(intrinsic) asthmatics. J Immunology 1997; 158:3539–3544.
32. Humbert M, Durham SR, Ying S, et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against ''intrinsic'' asthma being a distinct immunopathologic entity. Am J Respir Crit Care Med 1996; 154:1497–1504.
33. Corrigan CJ. Elevated interleukin-4 secretion by T lymphocytes: a feature of atopy or of asthma? [editorial]. Clin Exp Allergy 1995; 25:485–487.
34. Robinson D, Hamid Q, Ying S, et al. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5, and interferon-gamma cytokine gene expression. Am Rev Respir Dis 1993; 148:401–406.
35. Bentley AM, Hamid Q, Robinson DS, et al. Prednisolone treatment in asthma. Reduction in the number of eosinophils, T-cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon gamma cytokine gene expression within the bronchial mucosa. Am J Respir Crit Care Med 1996; 153:551–556.
36. Corrigan CJ, Brown PH, Barnes NC, Tsai JJ, Frew AJ, Kay AB. Glucocorticoid resistance in chronic asthma. Peripheral blood T lymphocyte activation and comparison of the T lymphocyte inhibitory effects of glucocorticoids and cyclosporin A. Am Rev Respir Dis 1991; 144:1026–1032.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

37. Leung DY, Martin RJ, Szefler SJ, et al. Dysregulation of interleukin 4, interleukin 5, and interferon gamma gene expression in steroid-resistant asthma. J Exp Med 1995; 181:33–40.
38. Louis R, Shute J, Biagi S, et al. Cell infiltration, ICAM-1 expression, and eosinophil chemotactic activity in asthmatic sputum. Am J Respir Crit Care Med 1997; 155: 466–472.
39. Robinson DS, Ying S, Bentley AM, et al. Relationships among numbers of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. J Allergy Clin Immunol 1993; 92:397–403.
40. Weller P. Updates on cells and cytokines. Human eosinophils. J Allergy Clin Immunol 1997; 100:283–287.
41. Teran L, Carroll M, Shute J, Holgate S. Interleukin-5 release into asthmatic airways 4 and 24 hours after endobronchial allergen challenge: its relationship with eosinophil recruitment. Cytokine 1999; 1:1–5.
42. Shalit M, Sekhsaria SM, Mauhorter S, Mahanti S, Malech HL. Early commitment to the eosinophil lineage by cultured human CD34+ cells: mRNA analysis. J Allergy Clin Immunol 1996; 98:344–354.
43. Sehmi R, Wood L, Watson R, et al. Allergen induced increased in IL-5Ra subunit expression on bone marrow derived CD34+ cells from asthmatic subjects. J Clin Invest 1997; 100:2466–2475.
44. Ying S, Robinson DS, Meng Q, et al. Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co- localization of eotaxin mRNA to bronchial epithelial and endothelial cells. Eur J Immunol 1997; 27:3507–3516.
45. Robinson D, Damia R, Zeibecoglou K, et al. CD34+/Interleukin-5Ra messenger RNA+ cells in the bronchial mucosa in asthma: potential airway eosinophil progenitors. Am J Respir Cell Mol Biol 1999; 20:9–13.
46. Rajakulasingam K, Till S, Ying S, et al. Increased expression of high affinity IgE (FcepsilonRI) receptor-alpha chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma. Am J Respir Crit Care Med 1998; 158:233–240.
47. Weller P, Lim K, Wan H, et al. Role of the eosinophil in allergic responses. Eur Respir J 1996; 9:109S–115S.
48. Metzger H. Molecular aspects of receptors and binding factors for IgE. Adv Immunol 1988; 43:277–312.
49. Galli SJ. Complexity and redundancy in the pathogenesis of asthma: reassessing the roles of mast cells and T cells. J Exp Med 1997; 186:343–347.
50. Humbert M, Grant JA, Taborda-Barata L, et al. High-affinity IgE receptor (FcepsilonRI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma. Am J Respir Crit Care Med 1996; 153:1931–1937.
51. Casale T, Wood D, Richardson H, et al. Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with methacholine bronchial hyperresponsiveness. J Clin Invest 1987; 79:1197–1203.
52. Church MK, Levi-Schaffer F. The human mast cell. J Allergy Clin Immunol 1997; 99:155–160.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

53. Liu F-T. Truly MASTerful cells: mast cells command B cell IgE synthesis. J Clin Inv 1997; 99:1465–1466.
54. Frandji P, Tkaczyk C, Oskeritizian C, David B, Desaymard C, Mecheri S. Exogenous and endogenous antigens are differentially presented by mast cells to CD4+ T lymphocytes. Eur J Immunol 1996; 26:2517–2528.
55. Rossi GL, Olivieri D. Does the mast cell still have a key role in asthma? Chest 1997; 112:523–529.
56. Macfarlane AJ, Kon OM, Smith SJ, Zeibecoglou K, Khan LN, Barata LT, McEuen AR, Buckley MG, Walls AF, Meng Q, Humbert M, Barnes NC, Robinson DS, Ying S, Kay AB. Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. J Allergy Clin Immunol 2000; 105(1 Pt 1):99–107.
57. Schroeder J. New concepts: the basophil. Updates on cells and cytokines. J Allergy Clin Immunol 1997; 99:429–433.
58. Guo C, Liu M, Galli S, Bochner B, Kagey-Sabotka A, Lichtenstein L. Identification of IgE-bearing cells in the late phase response to allergen in the lung as basophils. Am J Respir Cell Mol Biol 1994; 10(4):384–390.
59. MacGlashan DJ, White J, Huang S, Ono S, Schroeder J, Liechtenstein L. Secretion of IL-4 from human basophils: the relationship between IL-4 mRNA and protein in resting and stimulated basophils. J Immunol 1994; 152:3006–3016.
60. Li H, Sim T, Alam R. IL-13 released by and localised to human basophils. J Immunol 1996; 156(12):4833–4838.
61. Djukanovic R, Howarth P, Vrugt B, et al. Determinants of asthma severity. Int Arch Allergy Immunol 1995; 107:389.
62. Walker C, Kaegi MK, Braun P, Blaser K. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. J Allergol Clin Immunol 1991; 88:935–942.
63. Sont J, Han J, van Krieken J, et al. Relationship between the inflammatory infiltrate in bronchial specimens and clinical severity of asthma in patients treated with inhaled corticosteroids. Thorax 1996; 51:496–502.
64. Bradley B, Azzawi M, Jacobson M, et al. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. J Allergy Clin Immunol 1991; 88:661–674.
65. Wenzel SE, Szefler SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. Am J Respir Crit Care Med 1997; 156:737–743.
66. Holgate ST, EUROSCOP. Cellular composition of induced sputum in severe persistent asthma. Am J Respir Crit Care Med 1999; 159:A700.
67. Fahy JV, Kim KW, Liu J, Boushey HA. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. J Allergy Clin Immunol 1995; 95: 843–452.
68. Virchow JC, Jr., Walker C, Hafner D, et al. T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. Am J Respir Crit Care Med 1995; 151:960–968.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

69. Cox G. Glucocorticosteroid treatment inhibits apoptyosis in human neutrophils. J Immunol 1995; 154:4719–4725.
70. Linsley P, Ledbetter J. The role of CD28 receptor during T cell responses to antigen. Annu Rev Immunol 1993; 11:191–212.
71. Moller G, Overbeek S, Helden V, Meeuwsen C, et al. Increased numbers of dendritic cells in the bronchial mucosa of atopic asthmatic patients: downregulation by inhaled corticosteroids. Clin Exp Allergy 1996; 26:517–524.
72. Semper A, Hartley J. Dendritic cells in the lung-what is their relevence to asthma. Clin Exp Allergy 1996; 26:485–490.
73. Maurer D, Ebner C, Reininger B, Petzelbauer P, Fiebiger E, Stingl G. Mechanisms of Fc epsilon RI-IgE-facilitated allergen presentation by dendritic cells. Adv Exp Med Biol 1997; 417:175–178.
74. Maurer D, Ebner C, Reininger B, et al. The high affinity IgE receptor (FceR1) mediates IgE dependent allergen presentation. J Immunol 1995; 154:6285–6290.
75. Tunon-De-Lara JM, Redington AE, Bradding P, et al. Dendritic cells in normal and asthmatic airways: expression of the alpha subunit of the high affinity immunoglobulin E receptor (Fc epsilon RI-alpha). Clin Exp Allergy 1996; 26:648–655.
76. Gosset P, Lassalle P, Tonnel A, et al. Production of an interleukin-1 inhibitory factor by human alveolar macrophages from normals and allergic asthmatic patients. Am Rev Respir Dis 1988; 138:40–46.
77. Menz G, Ying S, Durham S, et al. Molecular concepts of IgE-mediated inflammation in atopic and non-atopic asthma. Allergy 1998; 53(Suppl 45):15–21.
78. Aubas P, Cosso B, Godard P, et al. Decreased suppressor cell activity of alveolar macrophages in bronchial asthma. Am Rev Respir Dis 1984; 130:875–878.
79. Holt P. Downregulation of immune responses in the lower respiratory tract: the role of alveolar macrophages. Clin Exp Immunol 1986; 63:261–270.
80. Lyons C, Ball E, Toews G, et al. Inability of human alveolar macrophages to stimulate resting T cells correlates with decreased antigen-specific T-cell macrophage binding. J Immunol 1986; 137(4):1173–1180.
81. Chelen C, Fang Y, Freeman G, et al. Human alveolar macrophages present antigen ineffectively due to defective expression of B7 costimulatory cell surface molecules. J Clin Invest 1995; 95:1415–1421.
82. Agea E, Forenza N, Piattoni S, et al. Expression of B7 costimulatory molecules and CD1a antigen by alveolar macrophages in allergic bronchial asthma. Clin Exp Allergy 1998; 28:1359–1367.
83. Robinson DS. T cell costimulation: a potential therapeutic target in asthma? [editorial]. Clin Exp Allergy 1998; 28:788–790.
84. Lenschow DJ, Walunas TL, Bluesone JA. CD28/B7 system of T cell costimulation. Ann Rev Immunol 1996; 14:233–258.
85. Bluestone J. Is CTLA4 a master switch for peripheral T cell tolerance? J Immunol 1997; 158:1989.
86. Schwartz R. Models of T cell anergy: Is there a common molecular mechanism? J Exp Med 1996; 184:1–8.
87. Keane-Myers AM, Gause WC, Finkelman FD, Xhou XD, Wills-Karp M. Development of murine allergic asthma is dependent upon B7-2 costimulation. J Immunol 1998; 160:1036–1043.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

88. Harris N, Peach R, Naemura J, Linsley PS, Le Gros G, Ronchese F. CD80 costimulation is essential for the induction of airway eosinophilia. J Exp Med 1997; 185: 177–182.
89. Jaffar Z, Roberts K, Pandit A, Djukanovic R, Holgate ST. B7 costimulation is required for IL-5 and IL-13 secretion by bronchial biopsy tissue of atopic asthmatic subjects in response to allergen stimulation. Am J Respir Cell Mol Biol 1999; 20: 153–162.
90. Hidi R, Riches V, Al-Ali M, et al. The role of B7-CD28/CTLA-4 costimulation and NF-kB in allergen-induced T cell chemotaxis by interleukin-16 and RANTES. J Immunology 2000; 164(1):412–418.
91. Larche M, Till SJ, Haselden BM, et al. Costimulation through CD86 is involved in airway antigen-presenting cell and T cell responses to allergen in atopic asthmatics. J Immunol 1998; 161:6375–6382.
92. Sayegh M. Finally, CTLA4Ig graduates to the clinic. J Clin Invest 1999; 103:1223–1225.
93. Cromwell O, Hamid Q, Corrigan CJ, et al. Expression and generation of interleukin-8, IL-6 and granulocyte- macrophage colony-stimulating factor by bronchial epithelial cells and enhancement by IL-1 beta and tumour necrosis factor-alpha. Immunology 1992; 77:330–337.
94. Laberge S, Ernst P, Ghaffar O, et al. Increased expression of interleukin-16 in bronchial mucosa of subjects with atopic asthma. Am J Respir Cell Mol Biol 1997; 17:193–202.
95. Kwon OJ, Jose PJ, Robbins RA, Schall TJ, Williams TJ, Barnes PJ. Glucocorticoid inhibition of RANTES expression in human lung epithelial cells. Am J Respir Cell Mol Biol 1995; 12:488–496.
96. Salvi SS, Semper A, Blomberg A, et al. Interleukin-5 production by human airway epithelial cells. Am J Respir Cell Mol Biol 1999; 20(5):984–991.
97. Lilly CM, Nakamura H, Kesselman H, et al. Expression of eotaxin by human lung epithelial cells: induction by cytokines and inhibition by glucocorticoids. J Clin Invest 1997; 99:1767–1773.
98. Aoki Y, Qiu D, Uyei A, Kao PN. Human airway epithelial cells express interleukin-2 in vitro. Am J Physiol 1997; 272:276–286.
99. Vignola A, Campbell A, Chanez P, et al. Activation by histamine of bronchial epithelial cells from non-asthmatic subjects. Am J Respir Cell Mol Biol 1993; 9: 411–417.
100. Van der Velden VH, Naber BA, Wierenga-Wolf AF, et al. Interleukin 4 receptors on human bronchial epithelial cells. An in vivo and in vitro analysis of expression and function. Cytokine 1998; 10:803–813.
101. Van der Velden VH, Naber BA, van der Spoel P, Hoogsteden HC, Versnel MA. Cytokines and glucocorticoids modulate human bronchial epithelial cell peptidases. Cytokine 1998; 10:55–65.
102. Nakamura Y, Azuma M, Okano Y, et al. Upregulatory effects of interleukin-4 and interleukin-13 but not interleukin-10 on granulocyte/macrophage colony-stimulating factor production by human bronchial epithelial cells. Am J Respir Cell Mol Biol 1996; 15:680–687.
103. Bayram H, Devalia JL, Sapsford RJ, et al. The effect of diesel exhaust particles

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

on cell function and release of inflammatory mediators from human bronchial epithelial cells in vitro. Am J Respir Cell Mol Biol 1998; 18:441–448.
104. Devalia JL, Campbell AM, Sapsford RJ, et al. Effect of nitrogen dioxide on synthesis of inflammatory cytokines expressed by human bronchial epithelial cells in vitro. Am J Respir Cell Mol Biol 1993; 9:271–278.
105. Walls A, Shute J, Warner J, et al. Mast cell and eosinophil proteases in the chronic allergic inflammatory response. In: Marone GAK, Lichtenstein L, Kay AB, Holgate ST, eds. Asthma, Physiology, Immunopharmacology and Treatment. San Diego: Academic Press, 1998:119–131.
106. Thompson P. Unique role of allergens and the epithelium in asthma. Clin Exp Allergy 1998; 28:110–118.
107. Wan H, Winton H, Soeller C, et al. Der p 1 facilitates transepithelial allergen delivery by dysruption of tight junctions. J Clin Invest 1999; 104:123–133.
108. Herbert C, King C, Ring P, Holgate S, et al. Augmentation of the permeability of the bronchial epithelium by the house dust mite, Der p 1. Am J Respir Cell Mol Biol 1995; 12:369–378.
109. Robinson C, Kalsheker N, Srinivasan N, et al. On the potential significance of the enzymatic activity of the mite allergens to immunogenicity. Clues to structure and function revealed by molecular characterisation. Clin Exp Allergy 1997; 27:10–21.
110. Campbell AM. Bronchial epithelial cells in asthma. Allergy 1997; 52:483–489.
111. Romagnani S, Parronchi P, D'Elios MM, et al. An update on human Th-1 and Th-2 cells. Int Arch Allergy Immunol 1997; 113:153–156.
112. Schleimer R, Sterbinsky S, Kaiser J, et al. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. J Immunol 1992; 148:1086–1092.
113. Naseer T, Minshall EM, Leung DY, et al. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. Am J Respir Crit Care Med 1997; 155:845–851.
114. Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. J Allergy Clin Immunol 1993; 92:313–324.
115. Pretolani M, Goldman M. IL-10: a potential therapy for allergic inflammation. Immunol. Today 1997; 18(6):277–280.
116. Robinson DS, Tsicopoulos A, Meng Q, Durham S, Kay AB, Hamid Q. Increased interleukin-10 messenger RNA expression in atopic allergy and asthma. Am J Respir Cell Mol Biol 1996; 14:113–117.
117. Cruikshank WW, Long A, Tarpy RE, et al. Early identification of interleukin-16 (lymphocyte chemoattractant factor) and macrophage inflammatory protein 1 alpha (MIP1 alpha) in bronchoalveolar lavage fluid of antigen-challenged asthmatics. Am J Respir Cell Mol Biol 1995; 13:738–747.
118. Borish L, Aarons A, Rumbyrt J, Cvietusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. J Allergy Clin Immunol 1996; 97:1288–1296.
119. Robinson DS, Tsicopoulos A, Meng Q et al. Increased IL-10 mRNA expression in atopic allergy and asthma. Am J Respir Cell Mol Biol 1996; 14:113–117.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

120. Ding L, Linsley P, Huang L, Germain R, Shevach I. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. J Immunol 1993; 151:1224–1234.
121. Borish L. IL-10: evolving concepts. J Allergy Clin Immunol 1998; 101:293–297.
122. Manetti R, Parronchi P, Giudizi M, et al. Natural killer cell stimulating factor (Interleukin-12) induces T helper type 1(Th)-specific immune responses and inhibits the development of IL-4 producing Th cells. J Exp Med 1993; 177:1199–1204.
123. Gavett SH, O'Hearn DJ, Li Z, et al. Interleukin-12 inhibits antigen-induced airway hyperresponsiveness, inflammation and Th2 cytokine expression in mice. J Exp Med 1995; 182:1527–1536.
124. Kiniwa M, Gately M, Gubler U, Chizzonite R, Fargeas C, Delespesse G. Recombinant interleukin-12 suppresses the synthesis of IgE by interleukin-4 stimulated human lymphocytes. J Clin Invest 1992; 90:262–266.
125. Okano Y, Nakamura Y, Sano T, Azuma M, Sone S. Interleukin-12 inhibits production of interleukin-5 but not of granulocyte macrophage-colony stimulating factor by antigen-stimulated blood mononuclear cells in allergic bronchial asthmatics. Int Arch Allergy Immunol 1998; 115:83–90.
126. Wills-Karp M. Interleukin-12 as a target for modulation of the inflammatory response in asthma. Allergy 1998; 53:113–119.
127. Bryan S, O'Connor BJ, Matti S, et al. Effects of recombinant-interleukin-12 on eosinophils, airway hyperreactivity and the late asthmatic response. Lancet 2000; 356:2149–2153.
128. Grunig G, Warnock M, Wakil A, et al. Requirement for IL-13 independent of IL-4 in experimental asthma. Science 1999; 282:2261–2263.
129. Wills-Karp M, Luyimbazi J, Xu X, et al. Interleukin-13: central mediator of allergic asthma. Science 1999; 282:2258–2261.
130. Zhu Z, Homer R, Wang Z, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiological abnormalities, and eotaxin production. J Clin Invest 1999; 103:779–788.
131. Kroegel C, Julius P, Matthys H, Virchow JC, Jr., Luttmann W. Endobronchial secretion of interleukin-13 following local allergen challenge in atopic asthma: relationship to interleukin-4 and eosinophil counts. Eur Respir J 1996; 9:899–904.
132. Defrance T, Carayon P, Billian G, et al. Interleukin-13 is a B cell stimulating factor. J Exp Med 1994; 179:135–143.
133. Bochner B, Klunk D, Sterbinsky S, et al. Interleukin-13 selectively induces vascular cell adhesion molecule-1 expression on human endothelial cells. J Immunol 1995; 154:799–803.
134. Humbert M, Durham SR, Kimmitt P, et al. Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and non-atopic subjects with asthma. J Allergy Clin Immunol 1997; 99:657–665.
135. Huang SK, Xiao HQ, Kleine-Tebbe J, et al. IL-13 expression at the sites of allergen challenge in patients with asthma. J Immunol 1995; 155:2688–2694.
136. Bellini A, Yoshimura H, Vittori E, Marini M, Mattoli S. Bronchial epithelial cells of patients with asthma release chemoattractant factors for T lymphocytes. J Allergy Clin Immunol 1993; 92:412–424.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

137. Center D, Kornfeld H, Cruikshank W. Interleukin 16 and its function as a CD4 ligand. Immunol Today 1996; 17(10):476–481.
138. Cruikshank W. Lymphocyte activation of T4+ lymphocytes and monocytes. J Immunol 1987; 138:3817–3825.
139. Okamura H, Tsutsi H, Komatsu T, et al. Cloning of a new cytokine that induces IFN-gamma production by T cells [see comments]. Nature 1995; 378:88–91.
140. Yoshimoto T, Okamura H, Tagawa YI, Iwakura Y, Nakanishi K. Interleukin 18 together with interleukin 12 inhibits IgE production by induction of interferon-gamma production from activated B cells. Proc Natl Acad Sci USA 1997; 94:3948–3953.
141. Barnes PJ, Adcock IM. Transcription factors and asthma. Eur Respir J 1998; 12: 221–234.
142. Barnes PJ, Adcock IM. NF-kappa B: a pivotal role in asthma and a new target for therapy. Trends Pharmacol Sci 1997; 18:46–50.
143. Barnes PJ, Adcock IM. Transcription factors and asthma. Eur Respir J 1998; 12: 221–234.
144. Nakamura Y, Ghaffar O, Olivenstein R, et al. Gene expression of the GATA-3 transcription factor is increased in atopic asthma. J Allergy Clin Immunol 1999; 103(2):215–222.
145. Leonardo M, Baltimore D. NF-kB: a pleitrophic mediator of inducible and tissue specific gene control. Cell 1989; 58:227–229.
146. Adcock I, Shirasaki H, Gelder C, et al. The effects of glucocorticoids on phorbol ester and cytokine stimulated transcription factor activation in human lung. Life Sci 1995; 55:1147–1153.
147. Xie Q, Kashiwabara Y, Nathan C. Role of transcription factor NF-kB Rel in induction of nitric oxide synthase. J Biol Chem 1994; 269:4705–4708.
148. Barnes PJ KM. Nuclear factor-κB—a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997; 336:1066–1071.
149. Barnes PJ, Adcock IM. Transcription factors. Clin Exp Allergy 1995; 25:46–49.
150. Barnes PJ, Pedersen S, Busse WW. Efficacy and safety of inhaled corticosteroids. Am J Respir Crit Care Med 1998; 157:S1–S35.
151. Adcock IM, Lane SJ, Brown CR, et al. Abnormal glucocorticosteroid receptor-activator protein 1 interaction in steroid resistant asthma. J Exp Med 1995; 182: 1951–1958.
152. Barnes PJ, Adcock I. Steroid resistance in asthma. Q J Med 1995; 88:455–468.
153. Brown P, Greville H, Finucane K. Asthma and irreversible airflow obstruction. Thorax 1980; 35:298–302.
154. Ebina M, Takahashi T, Chibita T, et al. Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma: a 3-D morphometric study. Am Rev Respir Dis 1993; 148(3):720–726.
155. Li X, Wilson J. Increased vascularity of the bronchial mucosa in mild asthma. Am J Respir Crit Care Med 1997; 156:229–233.
156. Takizawa T, Thurlsbeck W. Muscle and mucus gland size in the major bronchi of patients with chronic bronchitis, asthma, and asthmatic bronchitis. Am Rev Respir Dis 1971; 104:331–336.
157. Huber H, Koessler K. The pathology of bronchial asthma. Arch Int Med 1922; 30: 689–760.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

158. Roche W, Beasley R, Williams J, et al. Subepithelial fibrosis in the bronchi of asthmatics. Lancet 1989; 1(8637):520–524.
159. Brewster C, Howarth P, Djukanovic R, et al. Myofibroblasts and sub-epithelial fibrosis in bronchial asthma. Am J Respir Cell Mol Biol 1990; 3:507–511.
160. Redington A, Howarth P. Airway wall remodelling in asthma. Thorax 1997; 52: 310–312.
161. Redington A, Madden J, Frew A, et al. Transforming growth factor b1 in asthma-measurement in broncho-alveolar lavage fluid. Am J Respir Crit Care Med 1997; 156:642–647.
162. Minshall E, Leung D, Martin R, et al. Eosinophil associated TGF-b1 mRNA expression and airways fibrosis in bronchial asthma. Am J Respir Cell Mol Biol 1997; 17:326–333.
163. Ruoss SJ, Hartmann T, Caughey GH. Mast cell tryptase is a mitogen for culture fibroblasts. J Clin Invest 1991; 88:493–499.
164. Anwar A, Moqbel R, Walsh G, et al. Adhesion to fibronectin prolongs eosinophil survival. J Exp Med 1993; 177:839–843.
165. Georas S, McIntyre W, Ebisawa M. Expression of a functional laminin receptor a6b1 (VLA-6) on human eosinophils. Blood 1993; 82:2876–2879.
166. Goldring K, Warner J. Cell matrix interactions in asthma. Clin Exp Allergy 1997; 27:22–27.
167. Holgate S, Lackie P, Davies D, Roche W, Walls A. The bronchial epithelium as a key regulator of airway inflammation and remodelling in asthma. Clin Exp Allergy 1999; 29:90–95.
168. Polosa R, Krishna M, Howarth P, et al. Elevated epidermal growth factor receptor (EGFR) expression in bronchial epithelium of mild and severe asthmatics (abstr). Eur Respir J 1998; 12:265s.
169. Amishima M, Munakata M, Nasuhara Y, et al. Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway. Am J Respir Crit Care Med 1998; 157:1907–1912.
170. Puddicombe S, Holgate S, Davies D. Role of the epidermal growth factor receptor in epithelial repair in asthma. FASEB J 2000; 14(10):1362–1374.
171. Post M, Souza P, Liu J, et al. Keratinocyte growth and its receptor are involved in early lung branching. Development 1996; 122:3107–3115.
172. Finch P, Cunha G, Rubin J, Wong J, Ron D. Pattern of keratinocyte growth factor and KGF receptor expression during mouse fetal development suggests a role in mediating morphogenetic mesenchyme epithelial interactions. Dev Dyn 1995; 203: 223–240.
173. Redington A, Roche W, Madden J, et al. Basic fibroblast growth factor in asthma: measurement in bronchoalveolar lavage basally and following allergen challenge. J Allergy Clin Immunol 2001; 107(2):384–387.
174. Phunek P, Roche W, Turzikova J, Kurdmann J, Warner J. Eosinophilic inflammation in the bronchial mucosa of children with bronchial asthma (abstr). Eur Respir J 1997; 19:160s.
175. Djukanovic R, Wilson J, Britten K, et al. Effects of inhaled corticosteroids on airway inflammation and symptoms in asthma. Am Rev Respir Dis 1992; 145:669–674.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

176. Laitinen L, Laitinen A. Remodelling of asthmatic airways by glucocorticosteroids. J Allergy Clin Immunol 1996; 97:153–158.
177. British Thoracic Society NAC, Royal College of Physicians of London. The British guidelines on asthma management: 1995 review and position statement. Thorax 1997; 53:63–72.
178. Bentley AM, Menz G, Storz C, et al. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. Relationship to symptoms and bronchial responsiveness. Am Rev Respir Dis 1992; 146:500–506.
179. Walker C, Virchow JCJ, Bruijnzeel PL, Blaser K. T cell subsets and their soluble products regulate eosinophilia in allergic and nonallergic asthma. J Immunol 1991; 146:1829–1835.
180. Humbert M, Corrigan CJ, Kimmitt P, Till SJ, Kay AB, Durham SR. Relationship between IL-4 and IL-5 mRNA expression and disease severity in atopic asthma. Am J Respir Crit Care Med 1997; 156:704–708.
181. Poulter LW, Janossy G, Power C, Sreenan S, Burke C. Immunological/physiological relationships in asthma: potential regulation by lung macrophages. Immunol Today 1994; 15:258–261.
182. Humbert M, Ying S, Corrigan C, et al. Bronchial mucosal expression of the genes encoding chemokines RANTES and MCP-3 in symptomatic atopic and nonatopic asthmatics: relationship to the eosinophil-active cytokines interleukin (IL)-5, granulocyte macrophage-colony-stimulating factor, and IL-3. Am J Respir Cell Mol Biol 1997; 16:1–8.
183. Gosset P, Tillie-Leblond I, Oudin S, et al. Production of chemokines and proinflammatory and antiinflammatory cytokines by human alveolar macrophages activated by IgE receptors. J Allergy Clin Immunol 1999; 103:289–297.
184. Vercelli D, Jabara HH, Arai K, Geha RS. Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens. J Exp Med 1989; 169:1295–1307.
185. Gauchat JF, Lebman DA, Coffman RL, Gascan H, de Vries JE. Structure and expression of germline epsilon transcripts in human B cells induced by interleukin 4 to switch to IgE production. J Exp Med 1990; 172:463–473.
186. Punnonen J, Aversa G, Cocks BG, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc Natl Acad Sci USA 1993; 90:3730–3734.
187. Thyphronitis G, Tsokos GC, June CH, Levine AD, Finkelman FD. IgE secretion by Epstein-Barr virus-infected purified human B lymphocytes is stimulated by interleukin 4 and suppressed by interferon gamma. Proc Natl Acad Sci USA 1989; 86:5580–5584.
188. Jabara HH, Fu SM, Geha RS, Vercelli D. CD40 and IgE: synergism between anti-CD40 monoclonal antibody and interleukin 4 in the induction of IgE synthesis by highly purified human B cells. J Exp Med 1990; 172:1861–1864.
189. Wu CY, Sarfati M, Heusser C, et al. Glucocorticoids increase the synthesis of immunoglobulin E by interleukin 4-stimulated human lymphocytes. J Clin Invest 1991; 87:870–877.
190. Jabara HH, Ahern DJ, Vercelli D, Geha RS. Hydrocortisone and IL-4 induce IgE isotype switching in human B cells. J Immunol 1991; 147:1557–1560.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

191. Parronchi P, Tiri A, Macchia D, et al. Noncognate contact-dependent B cell activation can promote IL-4- dependent in vitro human IgE synthesis. J Immunol 1990; 144:2102–2108.
192. Gascan H, Gauchat JF, Roncarolo MG, Yssel H, Spits H, de Vries JE. Human B cell clones can be induced to proliferate and to switch to IgE and IgG4 synthesis by interleukin 4 and a signal provided by activated CD4+ T cell clones. J Exp Med 1991; 173:747–750.
193. Tsuyuki S, Tsuyuki J, Einsle K, Kopf M, Coyle AJ. Costimulation through B7-2 (CD86) is required for the induction of a lung mucosal T helper cell 2 (TH2) immune response and altered airway responsiveness. J Exp Med 1997; 185:1671–1679.
194. Bacharier LB, Jabara H, Geha RS. Molecular mechanisms of immunoglobulin E regulation. Int Arch Allergy Immunol 1998; 115:257–269.
195. Vercelli D, Jabara HH, Arai K, et al. Induction of IgE synthesis requires Interleukin-4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens. J Exp Med 1989; 169:1295–1307.
196. Coffman RL, Carty J. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. J Immunol 1986; 136:949–954.
197. Finkelman FD, Katona IM, Urban JF, Jr., Snapper CM, Ohara J, Paul WE. Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. Proc Natl Acad Sci USA 1986; 83:9675–9678.
198. Finkelman FD, Svetic A, Gresser I, et al. Regulation by interferon alpha of immunoglobulin isotype selection and lymphokine production in mice. J Exp Med 1991; 174:1179–1188.
199. Pene J, Rousset F, Briere F, et al. IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-gamma. J Immunol 1988; 141:1218–1224.
200. Lebman DA, Coffman RL. Interleukin 4 causes isotype switching to IgE in T cell-stimulated clonal B cell cultures. J Exp Med 1988; 168:853–862.
201. Yanagihara Y, Kajiwara K, Koshio T, et al. Production of IL-4 and expression of CD40 ligand by human CD8 T cells. J Allergy Clin Immunol 1999; 103:S405–S411.
202. Ryan JJ. Interleukin-4 and its receptor: essential mediators of the allergic response. J Allergy Clin Immunol 1997; 99:1–5.
203. Cocks BG, de Waal Malefyt R, Galizzi JP, de Vries JE, Aversa G. IL-13 induces proliferation and differentiation of human B cells activated by the CD40 ligand. Int Immunol 1993; 5:657–663.
204. Keegan AD, Zamorano J. Regulation of gene expression, growth, and cell survival by IL-4: contribution of multiple signaling pathways. Cell Res 1998; 8:1–13.
205. Li H, Sim TC, Alam R. IL-13 released by and localized in human basophils. J Immunol 1996; 156:4833–4838.
206. Pawankar R, Okuda M, Yssel H, Okumura K, Ra C. Nasal mast cells in perennial allergic rhinitics exhibit increased expression of the Fc epsilonRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells [see comments]. J Clin Invest 1997; 99:1492–1499.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

207. Punnonen JG, Aversa G, Cocks BG, et al. Interleukin 13 induces interleukin-4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc Natl Acad Sci USA 1993; 90:3730–3734.
208. McKenzie AN, Culpepper JA, de Waal Malefyt R, et al. Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function. Proc Natl Acad Sci USA 1993; 90:3735–3739.
209. Zurawski G, de Vries JE. Interleukin 13 elicits a subset of the activities of its close relative interleukin 4. Stem Cells (Dayt) 1994; 12:169–174.
210. Van der Pouw Kraan TC, Boeije LC, Troon JT, Rutschmann SK, Wijdenes J, Aarden LA. Human IL-13 production is negatively influenced by CD3 engagement. Enhancement of IL-13 production by cyclosporin A. J Immunol 1996; 156:1818–1823.
211. Arinobu Y, Atamas SP, Otsuka T, et al. Antagonistic effects of an alternative splice variant of human IL-4, IL- 4delta2, on IL-4 activities in human monocytes and B cells. Cell Immunol 1999; 191:161–167.
212. Mosley B, Beckmann MP, March CJ, et al. The murine interleukin-4 receptor: molecular cloning and characterization of secreted and membrane bound forms. Cell 1989; 59:335–348.
213. Idzerda RL, March CJ, Mosley B, et al. Human interleukin 4 receptor confers biological responsiveness and defines a novel receptor superfamily. J Exp Med 1990; 171:861–873.
214. Ryan DH, Nuccie BL, Ritterman I, Liesveld JL, Abboud CN. Cytokine regulation of early human lymphopoiesis. J Immunol 1994; 152:5250–5258.
215. Zurawski SM, Chomarat P, Djossou O, et al. The primary binding subunit of the human interleukin-4 receptor is also a component of the interleukin-13 receptor. J Biol Chem 1995; 270:13869–13878.
216. Rolling C, Treton D, Beckmann P, Galanaud P, Richard Y. JAK3 associates with the human interleukin 4 receptor and is tyrosine phosphorylated following receptor triggering. Oncogene 1995; 10:1757–1761.
217. Kohler I, Rieber EP. Allergy-associated I epsilon and Ec epsilon receptor II (CD23b) genes activated via binding of an interleukin-4-induced transcription factor to a novel responsive element. Eur J Immunol 1993; 23:3066–3071.
218. Shimoda K, van Deursen J, Sangster MY, et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature 1996; 380:630–633.
219. Palmer-Crocker RL, Hughes CC, Pober JS. IL-4 and IL-13 activate the JAK2 tyrosine kinase and Stat6 in cultured human vascular endothelial cells through a common pathway that does not involve the gamma c chain. J Clin Invest 1996; 98: 604–609.
220. Welham MJ, Learmonth L, Bone H, Schrader JW. Interleukin-13 signal transduction in lymphohemopoietic cells. Similarities and differences in signal transduction with interleukin-4 and insulin. J Biol Chem 1995; 270:12286–12296.
221. Wang LM, Keegan AD, Li W, et al. Common elements in interleukin 4 and insulin signaling pathways in factor-dependent hematopoietic cells. Proc Natl Acad Sci USA 1993; 90:4032–4036.
222. Burfoot MS, Rogers NC, Watling D, et al. Janus kinase-dependent activation of

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

insulin receptor substrate 1 in response to interleukin-4, oncostatin M, and the interferons. J Biol Chem 1997; 272:24183–24190.
223. Keegan AD, Nelms K, Wang LM, Pierce JH, Paul WE. Interleukin 4 receptor: signaling mechanisms. Immunol Today 1994; 15:423–432.
224. Deichmann K, Bardutzky J, Forster J, Heinzmann A, Kuehr J. Common polymorphisms in the coding part of the IL4-receptor gene. Biochem Biophys Res Commun 1997; 231:696–697.
225. Mitsuyasu H, Izuhara K, Mao XQ, et al. Ile50Val variant of IL4R alpha upregulates IgE synthesis and associates with atopic asthma [letter]. Nat Genet 1998; 19:119–120.
226. Van der Pouw Kraan TC, Van der Zee JS, Boeije LC, De Groot ER, Stapel SO, Aarden LA. The role of IL-13 in IgE synthesis by allergic asthma patients. Clin Exp Immunol 1998; 111:129–135.
227. Horner AA, Jabara H, Ramesh N, Geha RS. gamma/delta T lymphocytes express CD40 ligand and induce isotype switching in B lymphocytes. J Exp Med 1995; 181:1239–1244.
228. Worm M, Geha RS. CD40 ligation induces lymphotoxin alpha gene expression in human B cells. Int Immunol 1994; 6:1883–1890.
229. Rothe M, Sarma V, Dixit VM, Goeddel DV. TRAF2-mediated activation of NF-kappa B by TNF receptor 2 and CD40. Science 1995; 269:1424–1427.
230. Ishida TK, Tojo T, Aoki T, et al. TRAF5, a novel tumor necrosis factor receptor-associated factor family protein, mediates CD40 signaling. Proc Natl Acad Sci USA 1996; 93:9437–9442.
231. Ishida T, Mizushima S, Azuma S, et al. Identification of TRAF6, a novel tumor necrosis factor receptor-associated factor protein that mediates signaling from an amino-terminal domain of the CD40 cytoplasmic region. J Biol Chem 1996; 271: 28745–28748.
232. Faris M, Gaskin F, Geha RS, Fu SM. Tyrosine phosphorylation defines a unique transduction pathway in human B cells mediated via CD40. Trans Assoc Am Physicians 1993; 106:187–195.
233. Ren CL, Fu SM, Geha RS. Protein tyrosine kinase activation and protein kinase C translocation are functional components of CD40 signal transduction in resting human B cells. Immunol Invest 1994; 23:437–448.
234. Gordon J. CD23: novel disease marker with a split personality[comment]. Clin Exp Immunol 1991; 86:356–359.
235. Liu YJ, Cairns JA, Holder MJ, et al. Recombinant 25-kDa CD23 and interleukin 1 alpha promote the survival of germinal center B cells: evidence for bifurcation in the development of centrocytes rescued from apoptosis. Eur J Immunol 1991; 21:1107–1114.
236. Aubry JP, Pochon S, Graber P, Jansen KU, Bonnefoy JY. CD21 is a ligand for CD23 and regulates IgE production. Nature 1992; 358:505–507.
237. Sherr E, Macy E, Kimata H, Gilly M, Saxon A. Binding the low affinity Fc epsilon R on B cells suppresses ongoing human IgE synthesis. J Immunol 1989; 142:481–489.
238. Luo HY, Hofstetter H, Banchereau J, Delespesse G. Cross-linking of CD23 antigen by its natural ligand (IgE) or by anti-CD23 antibody prevents B lymphocyte proliferation and differentiation. J Immunol 1991; 146:2122–2129.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

239. Bonnefoy JY, Gauchat JF, Life P, Graber P, Mazzei G, Aubry JP. Pairs of surface molecules involved in human IgE regulation: CD23-CD21 and CD40-CD40L. Eur Respir J Suppl 1996; 22:63S–66S.
240. Fremeaux-Bacchi V, Fischer E, Lecoanet-Henchoz S, Mani JC, Bonnefoy JY, Kazatchkine MD. Soluble CD21 (sCD21) forms biologically active complexes with CD23: sCD21 is present in normal plasma as a complex with trimeric CD23 and inhibits soluble CD23-induced IgE synthesis by B cells. Int Immunol 1998; 10: 1459–1466.
241. Christie G, Barton A, Bolognese B, et al. IgE secretion is attenuated by an inhibitor of proteolytic processing of CD23 (Fc epsilonRII). Eur J Immunol 1997; 27:3228–3235.
242. Pene J, Rousset F, Briere F, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. Proc Natl Acad Sci USA 1988; 85:6880–6884.
243. Vercelli D, Jabara HH, Arai K, Yokota T, Geha RS. Endogenous interleukin 6 plays an obligatory role in interleukin 4-dependent human IgE synthesis. Eur J Immunol 1989; 19:1419–1424.
244. Jeppson JD, Patel HR, Sakata N, Domenico J, Terada N, Gelfand EW. Requirement for dual signals by anti-CD40 and IL-4 for the induction of nuclear factor-kappa B, IL-6, and IgE in human B lymphocytes. J Immunol 1998; 161:1738–1742.
245. Oshiba A, Gelfand EW. Engagement of the B-cell antigen receptor by antigen negatively regulates IgE production by antigen-specific B cells. J Allergy Clin Immunol 1999; 103:341–348.
246. Snapper CM, Paul WE. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science 1987; 236:944–947.
247. Del Prete G, Maggi E, Parronchi P, et al. IL-4 is an essential factor for for the IgE synthesis induced in vitro by human T cell clones and their supernatants. J Immunol 1988; 140:4193–4198.
248. Vercelli D, Jabara HH, Lauener RP, Geha RS. IL-4 inhibits the synthesis of IFN-gamma and induces the synthesis of IgE in human mixed lymphocyte cultures. J Immunol 1990; 144:570–573.
249. McLane MP, Haczku A, van de Rijn M, et al. Interleukin-9 promotes allergen-induced eosinophilic inflammation and airway hyperresponsiveness in transgenic mice. Am J Respir Cell Mol Biol 1998; 19:713–720.
250. Wang B, Rieger A, Kilgus O, et al. Epidermal Langerhans cells from normal human skin bind monomeric IgE via Fc epsilon RI. J Exp Med 1992; 175:1353–1365.
251. Gounni AS, Lamkhioued B, Delaporte E, et al. The high-affinity IgE receptor on eosinophils: from allergy to parasites or from parasites to allergy? J Allergy Clin Immunol 1994; 94:1214–1216.
252. Maurer D, Fiebiger E, Reininger B, et al. Expression of functional high affinity immunoglobulin E receptors (Fc epsilon RI) on monocytes of atopic individuals. J Exp Med 1994; 179:745–750.
253. Maurer D, Fiebiger S, Ebner C, et al. Peripheral blood dendritic cells express Fc epsilon RI as a complex composed of Fc epsilon RI alpha- and Fc epsilon RI gamma-chains and can use this receptor for IgE-mediated allergen presentation. J Immunol 1996; 157:607–616.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

254. Campbell AM, Vachier I, Chanez P, et al. Expression of the high-affinity receptor for IgE on bronchial epithelial cells of asthmatics. Am J Respir Cell Mol Biol 1998; 19:92–97.
255. Hasegawa S, Pawankar R, Suzuki K, et al. Functional expression of the high affinity receptor for IgE (FcepsilonRI) in human platelets and its' intracellular expression in human megakaryocytes. Blood 1999; 93:2543–2551.
256. Maurer D, Ebner C, Reininger B, et al. The high affinity IgE receptor (Fc epsilon RI) mediates IgE-dependent allergen presentation. J Immunol 1995; 154:6285–6290.
257. Shibaki A. FcepsilonRI on dendritic cells: a receptor, which links IgE mediated allergic reaction and T cell mediated cellular response. J Dermatol Sci 1998; 20: 29–38.
258. Kita H, Kaneko M, Bartemes KR, et al. Does IgE bind to and activate eosinophils from patients with allergy? J Immunol 1999; 162:6901–6911.
259. Bjerke T, Hoffmann HJ, Christensen EI, Poulsen LK, Skjold T, Dahl R. Regulation of FcepsilonARI synthesis in human eosinophils. Int Arch Allergy Immunol 1999; 118:440–442.
260. Blank U, Ra C, Miller L, White K, Metzger H, Kinet JP. Complete structure and expression in transfected cells of high affinity IgE receptor. Nature 1989; 337:187–189.
261. Repetto B, Bandara G, Kado-Fong H, et al. Functional contributions of the FcepsilonRIalpha and FepsilonRIgamma subunit domains in FcepsilonRI-mediated signaling in mast cells. J Immunol 1996; 156:4876–4883.
262. Metzger H. The high affinity receptor for IgE on mast cells. Clin Exp Allergy 1991; 21:269–279.
263. Ra C, Jouvin MH, Kinet JP. Complete structure of the mouse mast cell receptor for IgE (Fc epsilon RI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. J Biol Chem 1989; 264:15323–15327.
264. Alber G, Miller L, Jelsema CL, Varin-Blank N, Metzger H. Structure-function relationships in the mast cell high affinity receptor for IgE. Role of the cytoplasmic domains and of the beta subunit. J Biol Chem 1991; 266:22613–22620.
265. Hiraoka S, Furumoto Y, Koseki H, et al. Fc receptor beta subunit is required for full activation of mast cells through Fc receptor engagement. Int Immunol 1999; 11:199–207.
266. Padlan EA, Davies DR. A model of the Fc of immunoglobulin E. Mol Immunol 1986; 23:1063–1075.
267. Padlan EA, Helm BA. A modeling study of the alpha-subunit of human high-affinity receptor for immunoglobulin-E. Receptor 1992; 2:129–144.
268. Padlan EA, Helm BA. Modelling study of IgE/receptor interactions. Biochem Soc Trans 1993; 21:963–967.
269. Padlan EA, Helm BA. Modeling of the lectin-homology domains of the human and murine low-affinity Fc epsilon receptor (Fc epsilon RII/CD23). Receptor 1993; 3: 325–341.
270. Helm BA, Ling Y, Teale C, Padlan EA, Bruggemann M. The nature and importance of the inter-epsilon chain disulfide bonds in human IgE. Eur J Immunol 1991; 21: 1543–1548.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

271. Garman SC, Kinet JP, Jardetzky TS. The crystal structure of the human high-affinity IgE receptor (Fc epsilon RI alpha). Annu Rev Immunol 1999; 17:973–976.
272. Sayers I, Helm BA. The structural basis of human IgE-Fc receptor interactions. Clin Exp Allergy 1999; 29:585–594.
273. Sayers I, Cain SA, Swan JR, et al. Amino acid residues that influence Fc epsilon RI-mediated effector functions of human immunoglobulin E. Biochemistry 1998; 37:16152–16164.
274. McDonnell JM, Beavil AJ, Mackay GA, et al. Structure-based design of peptides that inhibit IgE binding to its high-affinity receptor Fc epsilon RI. Biochem Soc Trans 1997; 25:387–392.
275. Henry AJ, Cook JP, McDonnell JM, et al. Participation of the N-terminal region of Cepsilon3 in the binding of human IgE to its high-affinity receptor FcepsilonRI. Biochemistry 1997; 36:15568–15578.
276. Vercelli D, Helm B, Marsh P, Padlan E, Geha RS, Gould H. The B-cell binding site on human immunoglobulin E. Nature 1989; 338:649–651.
277. Robertson MW. Phage and *Escherichia coli* expression of the human high affinity immunoglobulin E receptor alpha-subunit ectodomain. Domain localization of the IgE-binding site. J Biol Chem 1993; 268:12736–12743.
278. Helm BA, Sayers I, Higginbottom A, et al. Identification of the high affinity receptor binding region in human immunoglobulin E. J Biol Chem 1996; 271:7494–7500.
279. Deisenhofer J. Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus* at 2.9- and 2.8-A resolution. Biochemistry 1981; 20:2361–2370.
280. Zheng Y, Shopes B, Holowka D, Baird B. Conformations of IgE bound to its receptor Fc epsilon RI and in solution. Biochemistry 1991; 30:9125–9132.
281. Beavil AJ, Young RJ, Sutton BJ, Perkins SJ. Bent domain structure of recombinant human IgE-Fc in solution by X-ray and neutron scattering in conjunction with an automated curve fitting procedure. Biochemistry 1995; 34:14449–14461.
282. Helm BA, Spivey AC, Padlan EA. Peptide blocking of IgE/receptor interaction: possibilities and pitfalls. Allergy 1997; 52:1155–1169.
283. Hamburger RN. Peptide inhibition of the Prausnitz-Kustner reaction. Science 1975; 189:389–390.
284. Beavil AJ, Beavil RL, Chan CM, et al. Structural basis of the IgE-Fc epsilon RI interaction. Biochem Soc Trans 1993; 21:968–972.
285. Nio N, Seguro K, Ariyoshi Y, et al. Inhibition of passive sensitization of human peripheral basophils by synthetic human immunoglobulin E peptide fragments. FEBS Lett 1993; 319:225–228.
286. Takemoto H, Nishimura S, Kosada Y, et al. Anti-human IgE monoclonal antibodies recognizing epitopes related to the binding sites of high and low affinity IgE receptors. Microbiol Immunol 1994; 38:63–71.
287. Presta L, Shields R, O'Connell L, et al. The binding site on human immunoglobulin E for its high affinity receptor. J Biol Chem 1994; 269:26368–26373.
288. Riske F, Hakimi J, Mallamaci M, et al. High affinity human IgE receptor (Fc epsilon RI). Analysis of functional domains of the alpha-subunit with monoclonal antibodies. J Biol Chem 1991; 266:11245–11251.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

289. Mallamaci MA, Chizzonite R, Griffin M, et al. Identification of sites on the human Fc epsilon RI alpha subunit which are involved in binding human and rat IgE. J Biol Chem 1993; 268:22076–22083.
290. Hulett MD, McKenzie IF, Hogarth PM. Chimeric Fc receptors identify immunoglobulin-binding regions in human Fc gamma RII and Fc epsilon RI. Eur J Immunol 1993; 23:640–645.
291. McDonnell JM, Beavil AJ, Mackay GA, et al. Structure based design and characterization of peptides that inhibit IgE binding to its high-affinity receptor. Nat Struct Biol 1996; 3:419–426.
292. Cook JP, Henry AJ, McDonnell JM, Owens RJ, Sutton BJ, Gould HJ. Identification of contact residues in the IgE binding site of human FcepsilonRIalpha. Biochemistry 1997; 36:15579–15588.
293. Bettler B, Texido G, Raggini S, Ruegg D, Hofstetter H. Immunoglobulin E-binding site in Fc epsilon receptor (Fc epsilon RII/CD23) identified by homolog-scanning mutagenesis. J Biol Chem 1992; 267:185–191.
294. Bonnefoy JY, Lecoanet-Henchoz S, Gauchat JF, et al. Structure and functions of CD23. Int Rev Immunol 1997; 16:113–128.
295. Yokota A, Kikutani H, Tanaka T, et al. Two species of human Fc epsilon receptor II (Fc epsilon RII/CD23): tissue-specific and IL-4-specific regulation of gene expression. Cell 1988; 55:611–618.
296. Yokota A, Yukawa K, Yamamoto A, et al. Two forms of the low-affinity Fc receptor for IgE differentially mediate endocytosis and phagocytosis: identification of the critical cytoplasmic domains. Proc Natl Acad Sci USA 1992; 89:5030–5034.
297. Abdelaziz MM, Devalia LJ, Khair OA, Calderon M, Sapsford RJ, Davis RJ. The effect of conditioned medium from cultures human bronchial epithelial cells on eosinophil and neutrophil chemotaxis and adherence in vitro. Am J Respir Cell Mol Biol 1995; 13:728–737.
298. Lee WT, Rao M, Conrad DH. The murine lymphocyte receptor for IgE. IV. The mechanism of ligand- specific receptor upregulation on B cells. J Immunol 1987; 139:1191–1198.
299. Kawabe T, Takami M, Hosoda M, et al. Regulation of Fc epsilon R2/CD23 gene expression by cytokines and specific ligands (IgE and anti-Fc epsilon R2 monoclonal antibody). Variable regulation depending on the cell types. J Immunol 1988; 141:1376–1382.
300. Denoroy MC, Yodoi J, Banchereau J. Interleukin 4 and interferons alpha and gamma regulate Fc epsilon R2/CD23 mRNA expression on normal human B cells. Mol Immunol 1990; 27:129–134.
301. Pochon S, Graber P, Yeager M, et al. Demonstration of a second ligand for the low affinity receptor for immunoglobulin E (CD23) using recombinant CD23 reconstituted into fluorescent liposomes. J Exp Med 1992; 176:389–397.
302. Alevy YG, Compas MB, Shaffer A, Rup B, Kahn LE. Regulation of human IgE synthesis by soluble factors. Papain treatment of a FcE receptor-positive B-cell line (RPMI-1788) releases regulatory factors for IgE synthesis. Int Arch Allergy Appl Immunol 1988; 86:125–130.
303. Saxon A, Ke Z, Bahati L, Stevens RH. Soluble CD23 containing B cell supernatants

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induce IgE from peripheral blood B-lymphocytes and costimulate with interleukin-4 in induction of IgE. J Allergy Clin Immunol 1990; 86:333–344.
304. Yu P, Kosco-Vilbois M, Richards M, Kohler G, Lamers MC. Negative feedback regulation of IgE synthesis by murine CD23. Nature 1994; 369:753–756.
305. Grangette C, Gruart V, Ouaissi MA, et al. IgE receptor on human eosinophils (FcERII). Comparison with B cell CD23 and association with an adhesion molecule. J Immunol 1989; 143:3580–3588.
306. Lecoanet-Henchoz S, Gauchat JF, Aubry JP, et al. CD23 regulates monocyte activation through a novel interaction with the adhesion molecules CD11b-CD18 and CD11c-CD18. Immunity 1995; 3:119–125.
307. Coyle AJ, Wagner K, Bertrand C, Tsuyuki S, Bews J, Heusser C. Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. J Exp Med 1996; 183:1303–1310.
308. Ikuta K, Takami M, Kim CW, et al. Human lymphocyte Fc receptor for IgE: sequence homology of its cloned cDNA with animal lectins. Proc Natl Acad Sci USA 1987; 84:819–823.
309. Beavil AJ, Edmeades RL, Gould HJ, Sutton BJ. Alpha-helical coiled-coil stalks in the low-affinity receptor for IgE (Fc epsilon RII/CD23) and related C-type lectins. Proc Natl Acad Sci USA 1992; 89:753–757.
310. Dierks SE, Barlett WC, Edmeades RL, Gould HJ, Rao M, Conrad DH. The oligomeric nature of the murine Fc epsilon RII/CD23. Implications for function. J Immunol 1993; 150:2372–2382.
311. Shi H, Qin S, Huang G, et al. Infiltration of eosinophils into the asthmatic airways caused by interleukin 5. Am J Respir Cell Mol Biol 1997; 16:220–224.
312. Stanworth DR, Humphrey JH, Bennich H, Johansson SG. Inhibition of Prausnitz-Kustner reaction by proteolytic-cleavage fragments of a human myeloma protein of immunoglobulin class E. Lancet 1968; 2:17–18.
313. Ishizaka K, Ishizaka T, Lee EH. Biologic function of the Fc fragments of E myeloma protein. Immunochemistry 1970; 7:687–702.
314. Dolovich J, Hargreave FE, Chalmers R, Shier KJ, Gauldie J, Bienenstock J. Late cutaneous allergic responses in isolated IgE-dependent reactions. J Allergy Clin Immunol 1973; 52:38–46.
315. Gordon J, Flores-Romo L, Cairns JA, et al. CD23: a multi-functional receptor/lymphokine? Immunol Today 1989; 10:153–157.
316. Kay AB. T lymphocytes and their products in atopic allergy and asthma. Int Arch Allergy Appl Immunol 1991; 94:189–193.
317. Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. J Allergy Clin Immunol 1982; 70:170–177.
318. Solley GO, Gleich GJ, Jordon RE, Schroeter AL. The late phase of the immediate wheal and flare skin reaction. Its dependence upon IgE antibodies. J Clin Invest 1976; 58:408–420.
319. Mudde GC, Van Reijsen FC, Boland GJ, de Gast GC, Bruijnzeel PL, Bruijnzeel-Koomen CA. Allergen presentation by epidermal Langerhans cells from patients with atopic dermatitis is mediated by IgE. Immunology 1990; 69:335–341.

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320. Leung DY. Mechanisms controlling the human immunoglobulin E response: new directions in the therapy of allergic diseases. Pediatr Res 1993; 33:S56–61; discussion S61–S62.
321. Corrigan CJ, Kay AB. T cells and eosinophils in the pathogenesis of asthma. Immunol Today 1992; 13:501–506.
322. Magnusson CG, Johansson SG. Clinical significance of anti-IgE autoantibodies and immune complexes containing IgE. Clin Rev Allergy 1989; 7:73–103.
323. Marone G, Spadaro G, Palumbo C, Condorelli G. The anti-IgE/anti-FcepsilonRIalpha autoantibody network in allergic and autoimmune diseases. Clin Exp Allergy 1999; 29:17–27.
324. Heusser C, Jardieu P. Therapeutic potential of anti-IgE antibodies. Curr Opin Immunol 1997; 9:805–813.
325. Boulet LP, Chapman KR, Cote J, et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response [see comments]. Am J Respir Crit Care Med 1997; 155:1835–1840.
326. Fahy JV, Fleming HE, Wong HH, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects [see comments]. Am J Respir Crit Care Med 1997; 155:1828–1834.
327. Fahy J, Cockcroft D, Boulet L, et al. Effect of aerosolized anti-IgE (E-25) on airway responses to inhaled allergen in asthmatic subjects. Am J Respir Crit Care Med 1998; 157:A410.
328. Kon OM, Kay AB. T cells and chronic asthma. Int Arch Allergy Immunol 1999; 118:133–135.
329. Kaminuma O, Mori A, Ogawa K, et al. Cloned Th cells confer eosinophilic inflammation and bronchial hyperresponsiveness. Int Arch Allergy Immunol 1999; 118:136–139.
330. Barnes PJ. Inhaled glucocorticoids for asthma. N Engl J Med 1995; 332:868–875.
331. Yang-Yen H, Chambard J, Sun Y. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of cDNA binding due to direct protein-protein interaction. Cell 1990; 62:1205–1215.
332. Schwiebert LM, Beck LA, Stellato C, et al. Glucocorticosteroid inhibition of cytokine production: relevance to antiallergic actions [published erratum appears in J Allergy Clin Immunol 1996 Sep;98(3):718]. J Allergy Clin Immunol 1996; 97: 143–152.
333. Stellato C, Beck LA, Gorgone GA, et al. Expression of the chemokine RANTES by a human bronchial epithelial cell line. Modulation by cytokines and glucocorticoids. J Immunol 1995; 155:410–418.
334. Schleimer R. Effects of glucocorticoids on inflammatory cells relevant to their therapeutic application in asthma. Am Rev Respir Dis 1990; 141:S59–S69.
335. Cronstein B, Kimmel S, Lenin R, Martiniuk F, Weissmann G. A mechanism for the anti-inflammatory effects of corticosteroids: the glucocorticoid receptor regulates leucocyte adhesion to endothelial cells and expression of endothelial-leucocyte adhesion molecule-1 and intercellular adhesion molecule-1. Proc Natl Acad Sci USA 1992; 89:9991–9995.
336. Adcock IM. Steroid resistance in asthma. Molecular mechanisms. Am J Respir Crit Care Med 1996; 154:S58–S61.

Marcel Dekker, Inc.
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337. Horwitz R, McGill K, Busse W. The role of leukotriene modifiers in the treatment of asthma. Am J Respir Crit Care Med 1998; 157:1363–1371.
338. Jardieu P. Anti-IgE therapy. Curr Opin Immunol 1995; 7:779–782.
339. Flores-Romo L, Shields J, Humbert Y, et al. Inhibition of an in vivo antigen-specific IgE response by antibodies to CD23. Science 1993; 261:1038–1041.
340. Kitani S, Kraft D, Fischler C, Mergenhagen SE, Siraganian RP. Inhibition of allergic reactions with monoclonal antibody to the high affinity IgE receptor [published erratum appears in J Immunol 1988; 141(2):709]. J Immunol 1988; 140:2585–2588.
341. Jardieu PM, Fick RB, Jr. IgE inhibition as a therapy for allergic disease. Int Arch Allergy Immunol 1999; 118:112–115.
342. Haba S, Nisonoff A. Inhibition of IgE synthesis by anti-IgE: role in long-term inhibition of IgE synthesis by neonatally administered soluble IgE. Proc Natl Acad Sci USA 1990; 87:3363–3367.
343. Kruse N, Tony HP, Sebald W. Conversion of human interleukin-4 into a high affinity antagonist by a single amino acid replacement. Embo J 1992; 11:3237–3244.
344. Aversa G, Punnonen J, Cocks BG, et al. An interleukin 4 (IL-4) mutant protein inhibits both IL-4 or IL-13- induced human immunoglobulin G4 (IgG4) and IgE synthesis and B cell proliferation: support for a common component shared by IL-4 and IL-13 receptors. J Exp Med 1993; 178:2213–2218.
345. Zurawski SM, Vega F, Jr., Huyghe B, Zurawski G. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. Embo J 1993; 12:2663–2670.
346. Carballido JM, Aversa G, Schols D, Punnonen J, de Vries JE. Inhibition of human IgE synthesis in vitro and in SCID-hu mice by an interleukin-4 receptor antagonist. Int Arch Allergy Immunol 1995; 107:304–307.
347. Atamas SP, Choi J, Yurovsky VV, White B. An alternative splice variant of human IL-4, IL-4 delta 2, inhibits IL-4-stimulated T cell proliferation. J Immunol 1996; 156:435–441.
348. Zhang JG, Hilton DJ, Willson TA, et al. Identification, purification, and characterization of a soluble interleukin (IL)-13-binding protein. Evidence that it is distinct from the cloned Il-13 receptor and Il-4 receptor alpha-chains. J Biol Chem 1997; 272:9474–9480.
349. Lock S, Kay A, Barnes N. Double-blind, placebo-controlled study of cyclosporin A as a corticosteroid-sparing agent in corticosteroid-dependent asthma. Am J Respir Crit Care Med 1996; 153:509–514.
350. Kon O, Sihra B, Compton C, Leonard T, Kay A, Barnes N. Randomised, dose ranging, placebo-controlled study of chimeric antibody to CD4 (keliximab) in chronic severe asthma. Lancet 1998; 352:1109–1113.
351. Abrams J, Lebwohl M, Guzzo C, et al. CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. J Clin Invest 1999; 103:1243–1252.
352. Mauser PJ, Pitman AM, Fernandez X, et al. Effects of an antibody to interleukin-5 in a monkey model of asthma. Am J Respir Crit Care Med 1995; 152:467–472.
353. Leckie M, ten Brinke A, Lordan J, et al. SB240563, A humanised anti-IL-5 monoclonal antibody. Single dose safety and activity in patients with asthma. Am J Respir Crit Care Med 1999; 159(3):A624.

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354. Bradding P, Roberts JA, Britten KM, et al. Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. Am J Respir Cell Mol Biol 1994; 10:471–480.
355. Broide D, Lotz M, Cuommo A, et al. Cytokines in the symptomatic asthmatic airways. J Allergy Clin Immunol 1992; 89:958–967.
356. Shah A, Church M, Holgate S. Tumor necrosis factor-alpha: a potential mediator of asthma. Clin Exp Allergy 1995; 25:1038–1044.
357. Schreiber S, Heinig T, Thiele H, Raedler A. Immunoregulatory role of IL-10 in patients with inflammatory bowel disease. Gastroenterology 1995; 108:1434–1444.
358. Stanford J, Stanford C. Immunotherapy of tuberculosis with *Mycobacterium vaccae* NCTC 11659. Immunobiology 1994; 191:555–563.
359. UCB Institute of Allergy, Brussels, Belgium. European White Paper on Allergy. Allergic Diseases as a Public Health Problem in Europe, 1996.
360. Obiri NI, Leland P, Murata T, Debinski W, Puri RK. The IL-13 receptor structure differs on various cell types and may share more than one component with IL-4 receptor. J Immunol 1997; 158(2):756–764.
361. Burastero SE, Magnani Z, Confetti C, Abbruzzesse L, Oddera S, Balbo P, Rossi GA, Crimi E. Increased expression of the CD80 accessory molecule by alveolar macrophages in asthmatic subjects and its functional involvement in allergen presentation to autologous Th2 lymphocytes. J Allergy Clin Immunol 1999; 103: 1136–1142.

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9

IgE-Mediated Response to Allergen in the Bronchoprovocation Laboratory

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I. Introduction

Standardized and controlled inhalation challenge with allergen has become a valuable laboratory tool in investigating the pathogenesis and pharmacotherapy of IgE-mediated asthmatic responses (1). This chapter reviews the history and science of standardized bronchoprovocation with allergen and the value of these tests in understanding the pathophysiology and treatment of asthma.

II. History

During the nineteenth century, allergic disease (allergic rhinitis, conjunctivitis, and asthma) was little recognized and poorly understood. In 1873, Blackley reported a series of investigations clearly documenting the role of pollen as a cause of allergic rhinitis, conjunctivitis, and asthma (2). This was almost a century before the humoral substance known as reagin, felt to be the cause of the allergic diathesis, was identified as a new serum immunoglobulin—IgE (3). Following the initial reports of bronchoprovocation with whole pollen grains in 1873, there appears to have been little activity in this regard until the late 1940s and 1950s (4–

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15). About this time, several investigators reported the use and value of allergen provocation with solubilized extracts of allergen. Endpoints were measured rather crudely by changes in vital capacity (4–10), by the appearance of symptoms and ''rough breathing'' (11,12), or by the *dose liminaire* causing a detectable change in the spirogram (14,15). Introduction of calculation of forced expiratory volume in one second (FEV_1 and FEV_1:forced vital capacity (FVC) ratio as a means of detecting airflow obstruction appeared about the mid-1950s. The FEV_1 soon became the major tool for the measurement of airway response to allergens as well as to nonallergic bronchoprovocation and to bronchodilators (13). The advantages of using FEV_1 over FVC are obvious in that the FEV_1 will fall substantially prior to a fall in vital capacity and, thus, provides earlier and safer documentation of airflow obstruction. The modern era of bronchoprovocation was established by two groups working independently, one headed by Professor Pepys in London (16–19) and the other by Professor Orie in The Netherlands (20–23). In the late 1960s and early 1970s, these two groups developed the basis for the current science of bronchoprovocation and outlined the effects of most of the common asthma pharmacotherapy.

III. Airway Response to Allergen

A. Early Asthmatic Response

The allergen-induced early asthmatic response (EAR) is both clinically and physiologically the most widely recognized allergen response. The allergen-induced EAR is an episode of airway obstruction with symptoms occurring immediately after allergen exposure or allergen provocation. Airflow obstruction is maximal between 10 and 20 (or 30) minutes after exposure and resolves spontaneously, usually within 90–120 minutes (1,16–23). This is the same time course as the well-recognized early cutaneous wheal-and-flare response to injected allergen. The presumed mechanism of the EAR is allergen-IgE–induced release of mast cell mediators, which then induce airway narrowing primarily from airway smooth muscle contraction. This is supported by observations that the airway response to allergen is dependent not only on the dose of allergen, but also upon the level of allergen-specific IgE (which should be a determinant of the amount of mediator release) and the degree of nonallergic airway hyperresponsiveness, which is a determinant of how responsive the airways will be to these released mediators (24,26).

The nonallergic airway responsiveness is generally measured with inhalation provocation tests with either histamine or methacholine. This relationship is strong enough that the dose of allergen required to produce a target EAR can be predicted, albeit crudely, by measurement of skin test endpoint (related to the level of allergen-specific IgE) and the provocation concentration of hista-

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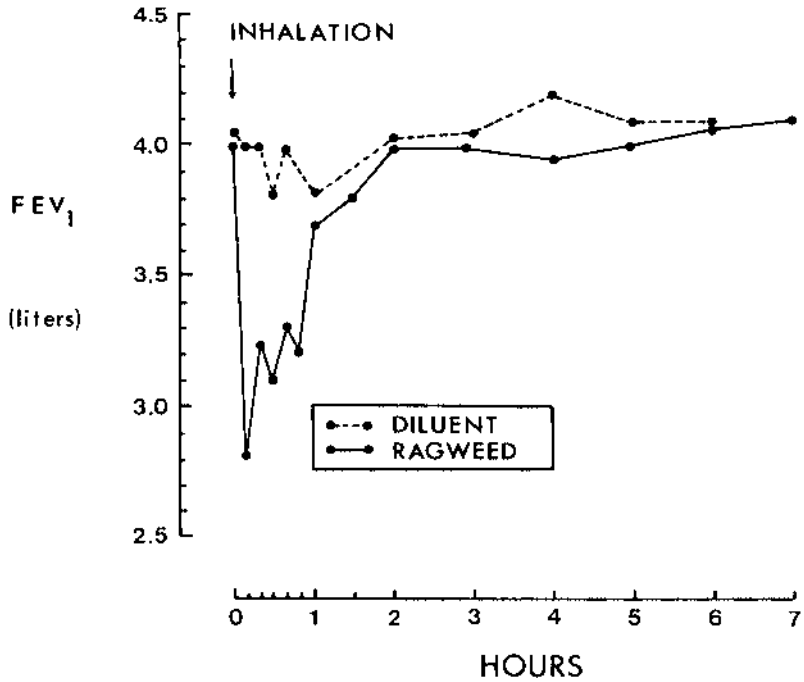

Figure 1 Allergen-induced isolated early asthmatic response (EAR). FEV_1 (L) is on the vertical axis and time (hr) post–allergen inhalation on the horizontal axis. Inhalation of ragweed allergen extract caused a 30% fall in FEV_1 maximal at 10 minutes and resolved by 2 hours (solid line); the response following inhaled diluent is shown in the dotted line.

mine or methacholine causing a 20% FEV_1 fall (methacholine PC_{20}) (27). The bronchospastic nature of the allergen-induced EAR is supported by the inhibition and reversal of the response by inhaled β_2-adrenoceptor agonists (1); these drugs may also influence the EAR prophylactically by preventing mast cell mediator release (28,29). An example of an allergen-induced early asthmatic response is shown in Figure 1.

B. Late Asthmatic Response

Blackley gave an elegant clinical description of a late asthmatic response (LAR) in 1873 following accidental heavy pollen exposure (2). The allergen-induced LAR in the laboratory was first identified in 1951 by Herxheimer (8) and has since been well characterized (16–24,30,31). The LAR is an episode of airflow obstruction beginning between 3 and 4 hours after allergen challenge and sponta-

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neous resolution of the EAR and lasting for 7–12 hours or more. Depending on the population studied and the allergen(s) used, the prevalence of LARs varies but is seen in about 50% of subjects with EAR. Isolated allergen-induced LARs are seen occasionally. The mechanism of the airflow obstruction and the immunopathogenesis of the LAR have been a matter of some controversy. Pepys described large allergen-induced LAR's following *Aspergillus* bronchoprovocation in subjects with allergic bronchopulmonary aspergillosis (17,19). These large LARs were poorly responsive to bronchodilator (19). Because of the time course, some of the pharmacological responsiveness, and the presence of serum precipitins against *Aspergillus*, Pepys suggested that a local Type III immune complex reaction was important in the pathogenesis of the LAR (17,19). By contrast, Orie and others argued that the appearance of LAR following bronchoprovocation with allergens such as pollen and house dust (mite), to which precipitating IgG antibodies could not be demonstrated, argued for IgE-dependent mechanisms as a cause of the LAR (23,31). The demonstration that polyclonal anaphylactogenic anti-IgE injected into the skin or inhaled into the airways could produce cutaneous late allergic responses (32) and airway late responses (33) gave strong support to the IgE dependence of the allergen-induced LAR.

The incomplete reversal of the large *Aspergillus*-induced LARs with sympathomimetic drug suggested that inflammation, either cellular or noncellular, might be an important component of the LAR. Although this hypothesis is likely correct, more recent studies have demonstrated that mild to moderate LARs are actually quite responsive to inhaled β_2-adrenoceptor agonists (34,35), and, thus, airway smooth muscle contraction plays a major role in the LAR.

The allergen-induced LAR is not as easy to predict as the EAR. In one study, the most important determinants of the LAR were a high level of allergen-specific IgE, a low dose of allergen causing a late cutaneous allergic response, and a large late cutaneous response in subjects with a small early cutaneous response to the same dose of allergen (36).

C. Allergen-Induced Increase in Airway Responsiveness

Allergen-induced increase in airway responsiveness is generally assessed by measuring airway responsiveness to histamine or methacholine. Altounyan was the first to report seasonal increases in airway responsiveness to histamine (37). This was first studied, using both histamine and methacholine, in the laboratory by Dr. Hargreave's group (38). In a series of studies, airway responsiveness to both histamine and methacholine was found to be increased both 7 and 24–30 hours post–allergen challenge, often lasting for several days (38–40) (see Fig. 2). This was seen in approximately 50% of positive allergen challenges (38) and correlated with the presence and size of the allergen-induced LAR (39) as well as with seasonal increases in airway responsiveness (40). Further investigations

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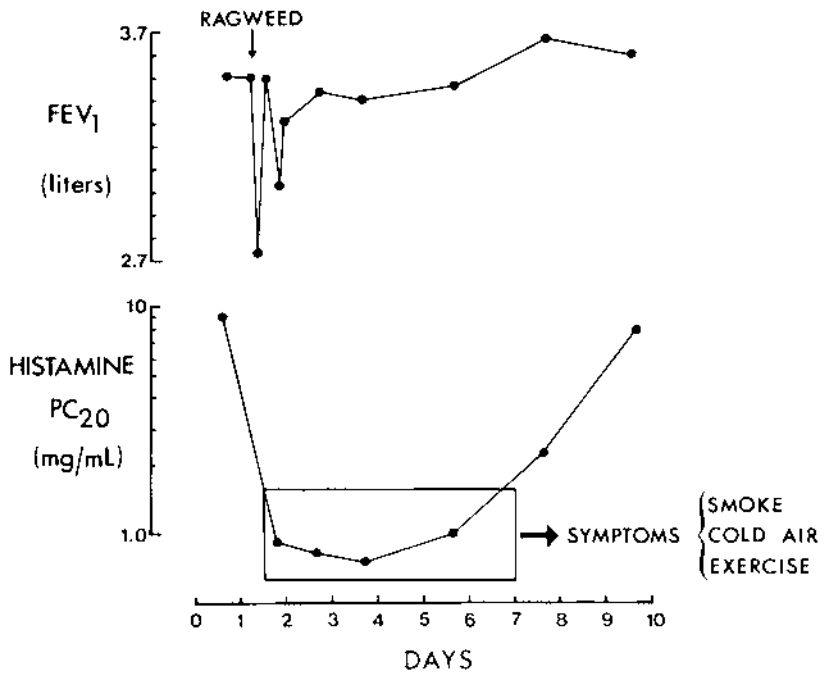

Figure 2 Allergen-induced increase in airway responsiveness to histamine. Vertical axis FEV_1 (L) on top graph and histamine PC_{20} (mg/mL, log scale) on bottom graph. Horizontal axis time in days following a single ragweed pollen extract inhalation. The top graph shows a spontaneously reversible dual asthmatic response. The bottom graph demonstrates allergen-induced increase in airway responsiveness to histamine; there was >10-fold fall in histamine PC_{20} lasting about 5 days postallergen. This was associated with reappearance of symptoms of asthma on exposure to ''nonallergic'' triggers. Histamine PC_{20} returned to baseline by 10 days. (From Ref. 122.)

have demonstrated that the airway responsiveness is not yet (much) increased 2 hours after allergen challenge (41) but does appear to be increased in the majority of subjects 3 hours after allergen challenge (42).

The mechanism of allergen-induced increase in airway responsiveness is uncertain. Animal studies suggest the need for polymorphonuclear leukocytes (43). The relationship of this to the LAR, the relationship of the LAR to allergen-induced airway inflammation (see below), and the correlation between airway responsiveness and airway eosinophilia (44) have led to the hypothesis that allergen-induced increase in airway responsiveness is somehow caused by airway inflammation. In fact, until recently, measurement of allergen-induced increases

in airway responsiveness was used as a surrogate measurement of allergen-induced inflammation (45).

Allergen-induced increase in airway responsiveness to methacholine is such a consistent feature of subjects with LARs that its measurement has become a routine component of allergen bronchoprovocation in many centers. Measurement of methacholine responsiveness at 3 hours may be convenient but may interfere with the onset of the LAR (42). Measurement at 7 hours may be difficult to interpret because of the LAR-related reduction in baseline FEV_1 (38). Therefore, this is generally measured by looking at a Δlog PC_{20} from 24 hours before allergen challenge to 24 hours after allergen challenge.

D. Allergen-Induced Airway Inflammation

Allergen-induced bronchial eosinophilia was first demonstrated by bronchoscopic bronchoalveolar lavage (BAL) in 1985 (46). Increase in BAL eosinophils was seen 6–7 hours after allergen challenge in subjects with an LAR but not at either 2 or 7 hours in subjects with an isolated EAR or at 7 hours in either normal or atopic subjects with a negative allergen challenge (46). This was confirmed with segmental allergen challenge and BAL (47). Until recently, assessment of airway inflammation has been considered too invasive to be a routine part of allergen challenges. However, standardized protocols are now available for hypertonic saline-induced sputum examination, and this may prove a useful routine addition to the allergen challenge (48).

IV. Standardization of Allergen Provocation Tests

A. Background

Allergen inhalation tests are primarily a research tool. For this reason, the principles of standardization have been developed to try and assure the best possible intraindividual reproducibility. The principles of standardization outlined below are based on both scientific and empirical concepts. Standardization encompasses technical aspects (aerosol generation, inhalation, measurement of response) and nontechnical or subject-related aspects, the latter designed to assure patient stability.

B. Technical Aspects

Technical aspects of standardization of allergen provocation tests are the same as for other inhalation provocation tests (49,50).

Aerosol Generation and Inhalation

Two major techniques of aerosol generation and inhalation are used for allergen provocation tests. The first is a 2-minute period of tidal breathing from a continu-

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ous output nebulizer—the technique used in our lab (27). The second involves a counted number (usually five) of inspiratory capacity breaths from a breath-acted dosimeter (49). The goal for standardization of all methods is to try to deliver a reproducible dose of allergen to the airways (50). A reliable nebulizer generating a reproducible aerosol whose operating characteristics are known and understood should be chosen. The nebulizer should be calibrated so that the output either per 2 minutes of tidal or per single breath-activated dose are known and kept constant. Since the primary goal in research allergen challenges is to minimize intraindividual variability, it is our practice to ensure that an identical nebulizer be used for repeat allergen challenges in the same individual. Attention should also be paid to the pattern of inhalation. This may be particularly important with regard to the five inspiratory capacity breaths technique. The speed and depth of inhalation may influence aerosol dose and deposition, and, indeed, we suspect it may influence the nebulizer output of some dosimeter systems. The use of and duration of a breathhold following inspiratory capacity breaths also has the potential to alter the retention of aerosol and therefore affect the response. It is therefore important to attempt to reproduce the inhalation pattern as closely as possible on repeat challenges. In this regard (as well as for measurement of the response), it can be very helpful if the same technician or research assistant performs all allergen challenges in the same individual.

Preparation of Allergen Extracts

Allergen extracts for inhalation provocation should be aqueous as opposed to glycerinated. Aqueous dilutions are made using a commercial diluent, for example, isotonic saline with 0.4% phenol and 0.03% human serum albumin added as preservatives and stabilizing agents. Allergen challenges are generally done with geometric (usually, but not always, doubling) increase in dose. Serial doubling dilutions are therefore made under the best sterile conditions. There are theoretical concerns regarding the stability of allergen preparations, particularly of weaker allergen dilutions, as well as the possibility of contamination. There are at least two ways to circumvent this concern. Perhaps the most common is to prepare a fresh batch of allergen dilutions, from the same stock solution, immediately prior to each allergen challenge. This is quite time-consuming. If a serial doubling dilution approach is used, even small errors will become multiplied with subsequent dilutions. An alternate approach which we have found effective is to prepare multiple single use aliquots of each allergen dilution specifically for an individual subject. These aliquots are then stored in the freezer in a vial that is compatible with our Wright nebulizer. They are thawed immediately prior to use and discarded after use. Since freezing and thawing of allergen extracts reduces their potency (personal observation), we always perform even the first allergen challenge with a set of previously frozen allergen extracts.

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Measurement of Response

Doubling doses of allergen are inhaled at 10- to 15-minute intervals (27,49,50). It is particularly important to start at a safe and low enough concentration so that there is no concern starting repeat allergen challenges at the same starting dose. Using the skin test endpoint (determined by duplicate prick skin tests with the same allergen and same dilutions to be used for inhalation) and a methacholine PC_{20}, it is possible to predict, usually within three doubling concentrations, the allergen PC_{20} (27). For safety sake, we start at least four concentrations below this prediction. If the first allergen challenge is an integral part of the study (e.g., a drug study), then it is ideal to reproduce the same challenge, the same starting dose, etc. for all subsequent challenges. By contrast, if the allergen challenge is a screening or dose finding challenge, and if many concentrations have to be administered in order to achieve a response, then it would be desirable to shorten subsequent challenges by starting two doubling doses below the responding dose (the goal being to administer at least three allergen concentrations) for each and every allergen challenge during a series. The response is generally measured by the FEV_1 using the baseline FEV_1 assessed after the subject has rested in the lab for 15–30 minutes. The best of three values is used as the baseline. Ten to 15 minutes after challenge (a time standardized for the specific technique used), the FEV_1 is repeated in duplicate at approximately 60-second intervals and the best of two acceptable maneuvers is retained. Doubling doses of allergen are administered until the FEV_1 has reached a target reduction generally of 15–20%; inhalations are then stopped. In subjects who have almost reached the target FEV_1 reduction, a number of options exist. The first is to repeat the last concentration rather than administer a doubling concentration. The second option is to wait a further 10 minutes to ascertain if the target FEV_1 reduction has been achieved and, if not, then to administer another inhalation either the same or a doubling dose depending on the FEV_1. Whatever approach is taken, the goal in subsequent allergen challenges should be to reproduce the pattern of dosing so long as it is possible to do so safely. Following completion of allergen inhalations, the FEV_1 is measured at 10- to 15-minute intervals for the first hour to determine the magnitude and pattern of the EAR. FEV_1 is usually determined again at 90 and 120 minutes and then hourly from 2–7 hours following allergen challenge (examples can be seen in Figs. 1 and 3). The EAR is usually measured as the maximum percent reduction in FEV_1 occurring during the first hour and the LAR as a maximum percent fall in FEV_1 between 2 or 3 and 7 or 8 hours after inhalation. Many investigators also measure the area under the curve (AUC) using a trapezoidal calculation. The EAR would thus be measured as the AUC from time 0–2 hours post–allergen inhalation and the LAR as the AUC from 3–7 hours post–allergen challenge. This approach, which is a same dose-variable response technique, is

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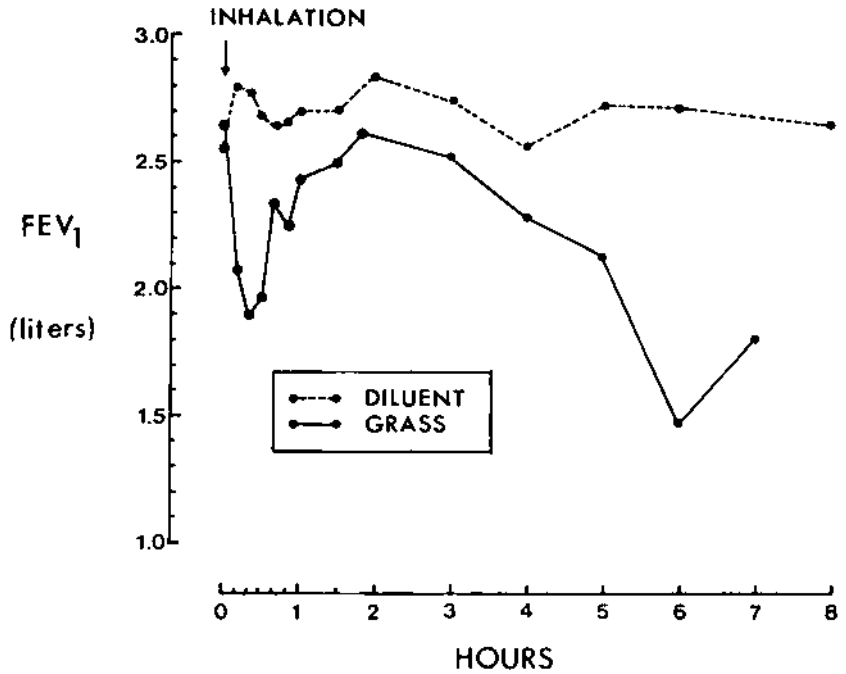

Figure 3 Allergen-induced dual asthmatic response. Legend as per Figure 1. Inhalation of grass pollen extract in this subject resulted in a 28% EAR maximal at 20 minutes with spontaneous resolution at 2 hours, followed by a late asthmatic response with a 45% fall in FEV_1 6 hours post–allergen inhalation. (From Ref. 122.)

commonly employed for allergen and exercise challenges (50). An alternate approach to reporting the response is to use a same response-variable dose technique such as that used to report the response to methacholine challenges (51). In this approach, the response (20% fall in FEV_1) is kept constant and the results reported as the dose or concentration producing this response either the provocative dose $(PD)_{20}$ or PC_{20} (or PC_{15}). We have successfully used the allergen PC_{15} in evaluating changes in EAR (52). An advantage of this approach is that it is no longer mandatory to administer the same dose of allergen in subsequent challenges; no matter how well controlled our subjects are, at least occasional subjects cannot achieve the same dose safely in repeat challenges. It is important in calculating and interpreting the allergen PC_{15} or PC_{20} that a rigorous doubling dosing schedule be administered without variations as described above. To this point, the allergen PC_{15} has only been used as a measure of the allergen-induced EAR.

Although this has not been addressed to our knowledge, it is conceivable that, in appropriate subjects, it would be possible to calculate an allergen PC_{15} for the late response (''LAR-PC_{15}'') as well.

Diluent Response

It is important to control allergen challenges with a diluent day during which diluent is inhaled in the same method that is used for allergen on three occasions at 10- to 15-minute intervals followed by identical monitoring of spirometry for 7 hours (1,27,31,50). The diluent day is generally performed the day before the first allergen challenge. This allows any early changes in FEV_1 following allergen to be differentiated from an irritant response (following diluent) and any late changes in FEV_1 following allergen to be differentiated from spontaneous diurnal fluctuations in FEV_1. The diluent day also allows the subject to become familiar with the procedure and gives time for performance of skin test endpoint and baseline methacholine challenge (27). Although it would probably be ideal if each subsequent allergen challenge were accompanied by a diluent day, in the interests of time, in subjects with stable FEV_1 following diluent, this is generally not repeated after the initial challenge.

C. Nontechnical Factors (Subject-Related)

Efforts should be made to assure that subjects undergoing allergen provocation are as stable as possible in order to have reproducible responses. This is at least as important as the technical factors, but we suspect it is not always as well appreciated as is standardization of technical factors. Important subject-related factors include stability in lung function (indeed stability in asthma control), medication use, and factors that may influence airway responsiveness.

Baseline FEV_1 should be reproducible within at least 10% prior to repeat allergen challenges. A case could be made for establishing stability of methacholine PC_{20} (within one doubling concentration) prior to repeat allergen challenges. The methacholine PC_{20} would have to be measured the day before adding to the time commitment; however, if determination of allergen-induced increase in airway responsiveness to methacholine is part of the protocol, then a preallergen methacholine PC_{20} is required.

Control of medications that might influence the response is also important (1,50). Bronchodilators (functional antagonists) that may inhibit the airway response must be withheld for their duration of action. This is most important for inhaled β_2-adrenoceptor agonists but is also relevant for other bronchodilating medications such as theophylline, leukotriene receptor antagonists (LTRAs), and anticholinergics. Anti-inflammatory medications (corticosteroids, cromones, LTRAs) ideally should not be used by patients undergoing allergen challenges. If the research protocol allows concomitant anti-inflammatories (or indeed requires

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concomitant anti-inflammatories), then it is important that they be used in a stable dose for many weeks (4–6 at least). The anti-inflammatory agent should be continued in the same dose throughout the study and should be withheld for 12 hours prior to challenge to avoid any direct inhibition of the allergen challenge. Ideally, subjects should be off all anti-inflammatory medications for 4–6 weeks prior to a study and for its duration. There are a number of nonasthma medications that should be considered and inquired about. Antihistamines may influence the allergen-induced asthmatic responses and should be withheld for their duration of action. Nonsteroidal anti-inflammatory drugs (aspirin, indomethacin, etc.) may influence certain aspects of the allergen-induced response and should be withheld. In fact, all medications, both prescription and proprietary, should be recorded in the subject's files. Attempts should be made to make sure that chronic use of such medications is kept the same both in type and dose. Allergen avoidance is also an important aspect of subject controls. Since allergen exposure may heighten airway response to methacholine (37–40), it has been empirically suggested that subjects for a series of allergen challenges should be tested at a time when there is no allergen exposure. This means strict avoidance of antigens that can be avoided (fur-bearing animals, etc.) and testing in a non-allergen season, i.e., the middle of winter. Unavoidable indoor allergens such as house dust mite may be considered to be a constant, but it is important to try and make sure that there are no major changes in exposure to these antigens. Subjects should not be pursuing new mite allergen exposure reduction strategies (or vice versa) while they are engaged in a series of allergen challenges.

Respiratory tract infections, particularly viral, may increase airway responsiveness (53), and it is recommended that allergen challenges be withheld for several weeks following a recent respiratory tract infection. There are some data to support enhancement of the LAR in subjects who have had a recent or a concurrent viral infection (54). It is our routine to avoid starting a study involving allergen challenges for at least 4 weeks following either allergen exposure or a respiratory tract infection. Development of a respiratory tract infection in the midst of an investigation may pose a problem. In crossover studies, it is possible and therefore desirable to postpone the next stage of the investigation until the respiratory tract infection has resolved and at least until the airway responsiveness has returned to baseline. In parallel studies this is not an option, and the study results will have to be interpreted in light of this, or the subject may even have to be dropped from the study.

D. Safety Concerns

Unlike histamine/methacholine challenges, allergen challenges are associated with a slight but significant risk of potentially serious adverse events (1). Physician attendance during the challenge and the EAR is mandatory. Facilities must

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be available for rapid reversal of bronchospasm and angioedema. Personnel trained in cardiopulmonary resuscitation and facilities in which to perform this must be available. Allergen challenges should probably be performed in or adjacent to a hospital. In some centers, patients have actually been hospitalized for the duration of an allergen challenge. There are also safety concerns related to the laboratory staff. We feel it is important to avoid laboratory staff being exposed to aerosol clouds of aqueous allergen droplets. In our lab, the Wright nebulizer is attached to the back of a Hans Rudolph valve and then via a mouthpiece to the patient. Two ventilator filters are placed on the expiratory line so as to capture the uninhaled aerosol and prevent exposure of lab staff.

E. Reproducibility

There are few data regarding reproducibility of allergen challenges. In one study, which addressed reproducibility of two challenges repeated at 2- to 12-week intervals using the 2-minute tidal doubling allergen dose challenge, satisfactory reproducibility was found (55). In 28 subjects with perhaps some selection bias, both the EAR and LAR were usually reproducible within what would be considered one doubling concentration (or the equivalent), i.e., a second EAR to the same concentration of allergen usually falls within the range of half the response to twice the response. If allergen PC_{20} were calculated, this would correspond to ± 1 doubling concentration. In this group of patients, the LAR was slightly more reproducible than the EAR. Using this repeatability data, fewer than eight subjects are required to have 90% power to show a clinically significant 50% reduction in the FEV_1 response during either the EAR or the LAR. Thus, the short-term reproducibility of EAR and LAR over a few weeks is generally good. However, despite adequate standardization, longer term reproducibility may not be as good and needs further assessment.

V. Allergen Provocation to Study Physiopathology of Asthma and Mechanisms of Action of Asthma Drugs

A. Study of Asthma Physiopathology

Airway inflammation, and probably its effects on airway structure (airway remodeling), are considered to be responsible not only for the acute but also the long-term changes in airway function in asthma such as persistent airway responsiveness and variable airflow obstruction (56–59).

Bronchoprovocation tests, particularly with allergens, have proved useful in exploring asthma physiopathology and in determining the characteristics and underlying mechanisms of the airway inflammatory process, as well as the influence of therapy on these processes (43,46,48,60–63). Allergen challenges have also been helpful in evaluating the relationships between exposure to allergen

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and the induced physiological and inflammatory changes in airways (25,39,46–48,64,65). The potential for allergen exposure to increase airway responses to a variety of stimuli, particularly when inducing a LAR, has been determined (39,41,64–66). Correlations between laboratory exposure and changes occurring after natural exposure to common allergens have further confirmed the validity of these tests as useful means for looking at asthma physiopathology (40).

Both whole-lung and endobronchial segmental allergen challenges have been used to investigate airway inflammatory events. Endobronchial challenges make it possible to evaluate the effects of different doses of allergen delivered to the lung segments while sampling a number of sites in the same subject (67). This technique, however, requires an endoscopy, is not readily repeatable, makes physiological correlates difficult, uses higher allergen doses that may not reflect real-life situations, and can induce severe bronchospasm. In spite of these drawbacks, however, the technique has proved valuable for confirming the findings of whole-lung challenges and exploring dose responses to allergen.

Allergen challenges in asthmatic subjects not using anti-inflammatory agents cause an airway inflammatory response characterized by an increase in the number and activation of such inflammatory cells in the airways as lymphocytes, mast cells, and eosinophils. There is also an increased production of inflammatory mediators such as cationic proteins, histamine, prostaglandins, and leukotrienes, as well as a variety of cytokines. The next sections provide examples of the findings reported.

Bronchoalveolar Lavage

Analysis of BAL fluid after allergen provocation showed an increase in the number of inflammatory cells such as eosinophils and CD8-positive T cells (46,68–70). Late-phase inflammatory events 6 hours after local allergen provocation involve the selective retention of airway T cells expressing specific cell-adhesion molecules (71). Levels of RANTES, MIP-1α, and MCP-1 were significantly increased in the BAL fluid obtained after endobronchial allergen challenge as compared with the saline control site, suggesting that they are involved in the regulation of cell traffic in asthma in response to allergen exposure (72).

Bronchial Biopsies

The development of techniques such as bronchial biopsies during endoscopic procedures made it possible to explore the effects of allergens on airway structure and components more thoroughly. In stable asthma, variable increases in eosinophils, mast cells, lymphocytes, and neutrophils in the airway wall have been observed in comparison with normal subjects, even when asthma was of recent onset or mild (57,73). After allergen challenges, bronchial biopsies have shown an increased expression of some adhesion molecules and an influx of mast cells,

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neutrophils, eosinophils, and CD3+ lymphocytes in the airways in the hours following allergen exposure (62,74,75).

Noninvasive Measurement of Airway Inflammation

Noninvasive techniques such as induced sputum analysis have been used to document airway inflammation after allergen exposure (48,76–79). Such techniques may be easily repeated, making it possible to correlate changes with physiological parameters over time.

Induced-sputum analysis was also able to demonstrate an increase in metachromatic cells and eosinophils after allergen inhalation (48,76). The late response to allergens was associated with an increased concentration of tumor necrosis factor α (TNFα) and interleukin 5 (IL-5) in induced sputum in addition to an increased eosinophil count and in eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO) (77). After aerosolized allergen challenge, ECP and histamine in induced-sputum supernatant were higher than at baseline (78). Tryptase was detectable in the sputum of many subjects.

Effects of Low-Level Exposure to Allergens

Recent studies have shown that even low levels of exposure to allergen can produce changes in airway function over time (79). This has important clinical relevance, as it may suggest that a subclinical exposure can be detrimental. In asthmatic subjects (79,80) and even in allergic nonasthmatic subjects (81), low-dose allergen exposure could induce airway inflammation and may possibly induce structural changes if persistent. Repeated inhalations of low doses of allergens caused an airway eosinophilia in atopic asthmatics and an increase in EG2-positive cells and IL-5, in association with an increase in airway responsiveness and mild worsening of asthma control (80). This occurred even without the development of acute bronchoconstriction or the development of a late asthmatic response.

Evaluation of the Airway Repair Process

Allergen challenges also make it possible to study the airway repair processes. Gizycki et al. obtained bronchial biopsies from allergic asthmatic patients and nonasthmatic control subjects 24 hours after diluent and allergen challenge (82). Light microscopy revealed statistically significant increases in the total number of inflammatory cells and in ''activated fibroblasts'' after allergen challenge. Electron microscopy showed significant increases in the total number of eosinophils and cells with the ultrastructural features of myofibroblasts after allergen challenge. The authors proposed that subjects who repeatedly develop a late response have increased numbers of migrating, contractile cells that may contribute to increase bronchial smooth muscle mass.

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More recently, the analysis of changes in matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in airway fluids after allergen challenges provided another means of studying the balance between airway inflammation, tissue damage, and changes in extracellular matrix proteins (83,84).

B. Mechanisms of Action of Asthma Drugs

Medications used to treat asthma include relievers, such as short-acting β_2-adrenoceptor agonists, to treat acute intermittent asthma symptoms. However, these drugs only provide acute symptomatic relief and do not influence asthma control or severity. In fact, the need for regular or frequent use is considered as evidence that asthma is not adequately controlled. Controllers (preventers) are ideal to prevent day- or nighttime asthma symptoms and optimize airway function. This last category includes anti-inflammatory medications such as inhaled and oral glucocorticosteroids, leukotriene receptor antagonists, and antiallergic or ''nonsteroidal'' agents such as cromoglycate and nedocromil. Controllers also include bronchodilators such as the long-acting β_2-adrenergic agents theophylline and ipratropium. Inhaled glucocorticosteroids are the first choice of this category of agents. The role of leukotriene receptor antagonists remains to be determined, but they offer a second choice for this purpose. When inhaled glucocorticosteroids are insufficient to control asthma, this may be achieved by adding drugs such as the long-acting inhaled β_2-adrenergic agents salmeterol or formoterol or the leukotriene receptors antagonists montelukast or zafirlukast (85,86). In a minority of patients there may be a role for other bronchodilators such as theophylline and ipratropium.

Various aspects of airway response may be used in different ways to predict the effects of both relievers or controllers on asthma. For example, the ability of relievers to inhibit EAR seems to be correlated with their efficacy in treating intercurrent asthma symptoms. In this regard, β_2-adrenoceptor agonists are the most powerful agents and the best choice for their rapid onset of action.

On the other hand, the inhibition of LAR and the allergen-induced increase in airway responsiveness is of particular interest in evaluating the efficacy of controllers and their mechanisms of action, particularly their anti-inflammatory effects. Many of them also block EAR, such as long-acting β_2-adrenoceptor agonists, theophylline, cromoglycate, nedocromil, 5 lipooxygenase inhibitors, and leukotriene receptor antagonists, while a single dose of inhaled corticosteroids (ICSs) is ineffective for this purpose (45,87–92) (Table 1). In contrast, treatment with corticosteroids ranging from one week to 2 months can result in a reduction of the EAR (93–95), partly because of the improvement in airway responsiveness, this last possibly related to a reduction in the number of airway inflammatory cells.

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Table 1 Influence of Antiasthmatic Drugs on Allergen-Induced Responses

	β_2-Agonists						
	4–8 hr[a]	≥12 hr[a]	Theophylline	Ipratropium	Inhaled steroids	Cromoglycate and nedocromil	LTRAs
Asthmatic responses							
Immediate	+++	+++	++	+	0[b]	+++	+++
Late	0[c]	+++	++	0	+++	+++	++
↑Airway responsiveness[d]	0	+++	0	0	+++	++	+
Allergen-induced airway inflammation	0	0/↑?	0/↓?	0	+++	+/++?	++?

±: Variable or doubtful effect; +: mild effect; ++: moderate effect; +++: excellent effect; ?: effect uncertain; 0: no effect.
LTRAs, Leukotriene receptor antagonists.
[a] Duration of action.
[b] +++ if done after regular treatment.
[c] Possible suppression if repeated administration of high doses.
[d] Suppression of increased bronchial reactivity secondary to inhalation of allergen.

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LAR can be inhibited by long-acting β_2-adrenoceptor agonists, cromoglycate, nedocromil, and a single dose of ICS administered either before the allergen challenge or in the interval phase between the EAR and LAR 2 hours after allergen challenge (29,96–99) (Table 1). Leukotriene-receptor antagonists may reduce EAR and LAR; however, inhibition of LAR by theophylline is controversial (86,100,101). Nedocromil sodium has proved effective in blocking the early and late asthmatic responses to allergens when given before, but it failed to reduce the fall in expiratory flows following allergen inhalation when given after the occurrence of the EAR (90).

EAR and LAR have been shown to be antagonized by long-acting β_2-adrenoceptor agonists such as salmeterol (96). Although we found what seemed to be an inhibitory effect of salmeterol on some markers of allergen-induced inflammation at 6 hours postexposure, we could not find such an effect 24 hours after exposure in patients who had been on this type of medication for a week and even observed a mild increase in some of these compared to placebo (102,103). This seems to be relatively similar to what had been reported with short-acting agents (104). However, such an increase was not found in another study using segmental challenge (105).

Allergen challenges have been used to evaluate the effects of new molecules such as anti-IgE antibody E25 (106) and IL-5 antagonists (107). The former was able to significantly inhibit allergen-induced responses, while the latter was not. Surprisingly, the latter was nevertheless able to reduce eosinophils in the blood and induced sputum, suggesting that the role of this cell in allergen-induced airway responses should be reassessed.

The determination of the inhibitory effect of drugs on allergen-induced responses, particularly LAR, has the potential to be specific to allergic asthma. This may explain why agents with what is considered mostly antiallergic properties such as cromoglycate and nedocromil are less useful in nonatopic asthma. This may also apply to new pharmaceuticals that operate via an IgE-specific pathway, although it has yet to be studied. Nevertheless, a majority of asthmatic sufferers have an allergic component that may contribute to the expression of the disease. Furthermore, agents such as corticosteroids seem to work as nonspecific blockers of inflammation, acting not only on allergen-related mechanisms but also on others.

VI. Predicting the Clinical Efficacy of Asthma Drugs from Their Inhibitory Effect on Allergen Bronchoprovocation

A. Asthma Outcomes: Control Versus Severity of Asthma

The goals of asthma therapy are to achieve optimal asthma control and reduce the severity of the disease while avoiding untoward side effects of the medication

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(85,86). Optimal control of asthma, defined by the absence of respiratory symptoms, no need for rescue bronchodilator, and a normal pulmonary function, may be difficult to achieve. However, most asthmatic patients can obtain acceptable control, with daytime symptoms fewer than 4 days a week, nighttime symptoms less often than once a week, mild and infrequent exacerbations, normal physical activities, no absenteeism at work, no need for regular rescue inhaled short-acting β_2-adrenoceptor agonist, FEV_1, or peak expiratory flows (PEF) ideally $\geq$90% personal best and PEF diurnal variation of $<$15% (85). Acceptable control is obtained by taking appropriate environmental measures, ensuring proper education of patients, and prescribing individualized pharmacotherapy; level of control should be regularly assessed and the treatment adjusted accordingly.

The initial measure of severity of asthma in a patient is based on the frequency and duration of respiratory symptoms and the degree of airflow limitation. Once asthma is well controlled, one of the best ways to judge severity is to determine the level of treatment needed to maintain acceptable control. Signs of severe or poorly controlled asthma also include a prior near-fatal episode, recent hospitalization, or visit to the emergency room; nighttime symptoms; limitation of daily activities; need for inhaled β_2-adrenoceptor agonist several times per day or at night; and FEV_1 or PEF of $<$60% of predicted value.

B. Correlations Between Inhibitory Effects of Asthma Drugs on Allergen Challenge and Their Clinical Efficacy

Allergen challenges have been used as a method for evaluating the relative potency of asthma drugs, particularly anti-inflammatory agents. Asthma is considered to be an inflammatory disorder, and the ideal agent for long-term treatment of this disease is the one that can best antagonize the inflammatory process while producing no significant untoward side effects. Studies have indeed shown anti-inflammatory treatment to be associated with improved asthma control, reduced morbidity and mortality, and reduced health-care costs (108–110). As previously mentioned, the first studies looked at the effects of such bronchodilators as ipratropium on allergen-induced responses; later studies looked at others agents, such as β_2-adrenoceptor agonists, cromoglycate, beclomethasone, and others.

Drugs that help control asthma and act as good maintenance therapy generally reduced the allergen-induced responses, including the late asthmatic response, while agents with minimal effect on these last, such as calcium channel blockers and antihistamines, were not found useful for this purpose (111,112).

ICSs are the mainstay of asthma treatment. In search of a method for determining the bioequivalence of these preparations, researchers have assessed their inhibitory effect on allergen-induced LAR and confirmed their ability to attenuate both the LAR and the secondary increase in airway responsiveness (113,114).

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The single-dose ICS/allergen inhalation model has been used recently both to look at the comparative efficacy of ICS and as a predictor of clinical efficacy of different doses of these agents (115). The efficacy of budesonide, a clinically useful agent, was tested at doses of 200 and 800 μg and compared with the efficacy of a poorly effective ICS (D-5159) at a dose of 8 mg and a placebo. Both budesonide doses, but not the D-5159, produced a significant inhibition of the LAR. The allergen-induced LAR model seemed therefore able to differentiate a single dose of an active inhaled corticosteroid from a placebo and a highly potent ICS from a weak one. However, the model did not differentiate between two fourfold doses of the active ICS. This is in keeping with other observations showing marked efficacy of the inhaled corticosteroid used in reducing allergen-induced LAR but little dose-response relationship, suggesting that the dose response had plateaued at low doses in these subjects. This may reflect either the excellent anti-inflammatory effect of those agents, even at low doses, or the patients' narrow range of asthma severity, being very mild in all those tested.

VII. Allergen Provocation and the Development of New Therapeutic Agents

A. Allergen Challenges as Early Assessment of Usefulness of Drugs

Inhalation of allergen in the laboratory can be useful to study the effects and properties of these newly developed asthma medications. With techniques such as bronchial biopsies, BAL, and noninvasive techniques for assessing airway inflammation, the allergen bronchoprovocation has become a powerful test to compare the anti-inflammatory properties of agents. Allergen exposure in sensitized subjects is one of the most frequent triggers of airway inflammation. To be effective in controlling asthma, as a large majority of asthmatic patients have an allergic component, the drug should be able to antagonize the inflammatory response to current allergen exposure. But this may not be the only reason of their efficacy as they may also antagonize baseline airway inflammation and responsiveness or the one induced by triggers other than allergens. If a new agent is developed, it would be useful to compare its potential to inhibit the different components of the airway inflammatory response with standard therapies such as ICSs. As discussed later, some agents may help to control asthma without acting on inflammatory responses. The evaluation of mechanisms by which new agents inhibit allergen-induced responses should therefore be included, not only clinical or physiological measures. Theoretically, it could also help to determine the magnitude of the anti-inflammatory effect if, for example, we were to determine the percent reduction in some parameters such as eosinophils in sputum.

Furthermore, we are just beginning to explore the potential effects of allergen exposure on airway remodeling, and although noninvasive techniques for

looking at these aspects remain to be developed, there is a potential to look at this aspect of the response to the allergens in the future.

Finally, it is quite possible that earlier use of agents acting on allergen-induced airway responses could prevent the development of asthma or help to prevent asthma from becoming more severe or refractory following allergen exposure. Adequate environmental control is often difficult to achieve, and there may be a role, at least in targeted groups such as highly atopic subjects or those with asymptomatic airway responsiveness, for early anti-inflammatory treatment at the very onset or maybe even before asthma becomes symptomatic (116,117). This should be further explored.

B. Pitfalls

The correlation between the anti-inflammatory properties of asthma drugs and their potential for inhibiting allergen-induced airway physiological responses has not always been very good, supporting the need for additional assessment of airway inflammation. Indeed, although it was initially thought that drugs that could inhibit the late asthmatic responses to allergens probably had clinically relevant anti-inflammatory properties, it was shown that this was not always the case. For example, long-acting β_2-adrenoceptor agonists were initially considered likely to have some significant airway anti-inflammatory effect and to be able to block the EAR and LAR for a prolonged period of time (118). Further studies showed that they had no significant effect on the airway inflammatory process, at least in regard to cellular infiltration and some mediators release (101,118–120). In this case, the protection against the allergen-induced responses was mostly due to the functional antagonist effect of the drug rather than the inhibition of the allergen-induced airway inflammation. It is obvious that mechanisms other than anti-inflammatory effects can prevail in this inhibition, as it may be obtained by agents without recognized anti-inflammatory effect such as β_2-adrenoceptor agonists or furosemide (121).

VIII. Conclusion

Allergen bronchoprovocation has been most useful in the investigation of the pathophysiology of asthma and in defining the clinical efficacy and the properties of asthma medications, particularly in their potential to block various aspects of airway inflammation. A correlation between the clinical efficacy of a drug and its potential for suppression of allergen-induced responses, particularly LAR, has been observed, although the mechanisms by which this occurs, either a functional antagonism or an anti-inflammatory effect, should be documented. Drugs that do not antagonize allergen-induced responses will unlikely be suitable for maintenance treatment of asthma. The dose response to agents such as ICSs is relatively

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flat, however, showing the sensitivity of allergen responses to be inhibited by low doses of these agents. Furthermore, the use of allergen-induced airway repair responses, considered to play a role in long-term outcome of asthma, are just beginning to be evaluated, and the effects of drugs on this process remain to be determined.

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References

1. Cockcroft DW. Bronchial inhalation tests II: measurement of allergic (and occupational) bronchial responsiveness. Ann Allergy 1987; 59:89–99.
2. Blackley CH. In: Experimental Researches on the Cause and Nature of Catarrhus Aestivus (Hay-Fever or Hay-Asthma). London: Balliere Tindall & Cox, 1873. Reprinted by Dawson's of Pall Mall, 1959.
3. Ishizaka K, Ishizaka T, Hornbrook MM. Physiochemical properties of reaginic antibody; V. Correlation of reaginic activity with E-globulin antibody. J Immunol 1966; 97:840–853.
4. Lowell FC, Schiller IW. Measurement of changes in vital capacity as a means of detecting pulmonary reactions to inhaled aerosolized allergenic extracts in asthmatic subjects. J Allergy 1948; 19:100–107.
5. Lowell FC, Schiller IW. The induction of asthma-like attacks in subjects with ''idiopathic'' asthma. J Allergy 1948; 19:172–177.
6. Herxheimer H. Induced asthma in man. Lancet 1951; 260:1337–1341.
7. Herxheimer H. Bronchial obstruction induced by allergens, histamine and acetyl-beta-methylcholinechloride. Int Arch Allergy 1951; 2:27–39.
8. Herxheimer H. The late bronchial reaction in induced asthma. Int Arch Allergy Appl Immunol 1952; 3:323–333.
9. Feinberg SM, Stier RA, Grater WC. A suggested quantitative evaluation of the degree of sensitivity of patients with ragweed pollinosis. J Allergy 1952; 23:387–394.
10. Schiller IW, Lowell FC. The effect of drugs in modifying the response of asthmatic subjects to inhalation of pollen extracts as determined by viral capacity measurements. Ann Allergy 1947; 5:564–566.
11. Colldahl H. A study of provocation tests on patients with bronchial asthma: I. The reliability of provocation tests performed under different conditions. Acta Allergol 1952; V:133–142.
12. Colldahl H. A study of provocation tests on patients with bronchial asthma: II. The outcome of provocation tests with different antigens. Acta Allergol 1952; V:143–153.

13. Citron KM, Frankland AW, Sinclair JD. Inhalation tests of bronchial hypersensitivity in pollen asthma. Thorax 1958; 13:229–232.
14. Tiffeneau R. Recherches quantitatives sur les mediateurs bronchoconstrictifs produits par inhalation continue d'allergenes. Path Biol 1959; 21–22:2293–2310.
15. Tiffeneau R. Amples variations du degre de l'allergie pulmonaire produites par administration d'llergenes par voie respiratoire. Int Arch Allergy 1960; 17:193–210.
16. Pepys J, Chan M, Hargreave FE, McCarthy DS. Inhibitory effects of disodium cromoglycate on allergen-inhalation tests. Lancet 1968; 2:134–137.
17. Pepys J. Immunological mechanisms in asthma. In: Porter R, Birch J, eds. Identification of Asthma. London: Churchill Livingstone, 1971:87–97.
18. Pepys J, Davies RJ, Breslin ABX, Hendricks DJ, Hutchcroft BJ. The effects of inhaled beclomethasone dipropionate (Becotide) and sodium cromoglycate on asthmatic reactions to provocation tests. Clin Allergy 1974; 4:13–24.
19. Pepys J, Hutchcroft BJ. Bronchial provocation tests in etiologic diagnosis and analysis of asthma. Am Rev Respir Dis 1975; 112:829–859.
20. Van Lookeren Campagne JG, Knol K, deVries K. House dust provocation in children. Scand J Respir Dis 1969; 50:76–85.
21. Booij-Noord H, Orie NGM, deVries K. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J Allergy Clin Immunol 1971; 48:344–354.
22. Booij-Noord H, deVries K, Sluiter HJ, Orie NGM. Late bronchial obstructive reaction to experimental inhalation of house dust extract. Clin Allergy 1972; 2:43–261.
23. Orie NGM, Van Lookeren Campagne JG, Knol K, Booij-Noord H, deVries K. Late reactions in bronchial asthma. In: Pepys J, Yamamura I, eds. Intal in Bronchial Asthma. Tokyo: Proceedings of the 8th International Congress of Allergollogy, 1974:17–29.
24. Killian D, Cockcroft DW, Hargreave FE, Dolovich J. Factors in allergen-induced asthma: relevance of the intensity of the airways allergic reaction and non-specific bronchial reactivity. Clin Allergy 1976; 6:219–225.
25. Cockcroft DW, Ruffin RE, Frith PA, Cartier A, Juniper EF, Dolovich J, Hargreave FE. Determinants of allergen-induced asthma: dose of allergen, circulating IgE antibody concentration and bronchial responsiveness to inhaled histamine. Am Rev Respir Dis 1979; 120:1053–1058.
26. Nathan RA, Kinsman RA, Spector SL, Horton DJ. Relationship between airway response to allergens and nonspecific bronchial reactivity. J Allergy Clin Immunol 1979; 64:491–499.
27. Cockcroft DW, Murdock KY, Kirby J, Hargreave FE. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. Am Rev Respir Dis 1987; 135:264–267.
28. Church MK, Young KD. The characteristics of inhibition of histamine release from human lung fragments by sodium cromoglycate, salbutamol, and chlorpromazine. Br J Pharmacol 1983; 78:671–679.
29. Howarth PH, Durham SR, Lee TH, Kay B, Church MK, Holgate ST. Influence on albuterol, cromolyn sodium, and ipratropium bromide on the airway and circulating

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mediator responses to allergen bronchial provocation in asthma. Am Rev Respir Dis 1985; 132:986–992.
30. Burtin PP, Buffe D, Garton J, Dominjon-Monnier F, Kourilsky R. L'allergie retardée à la poussière de maison. Int Arch Allergy 1962; 21:8–19.
31. Robertson DG, Kerigan AT, Hargreave FE, Chalmers R, Dolovich J. Late asthmatic responses induced by ragweed pollen allergen. J Allergy Clin Immunol 1974; 54: 244–254.
32. Dolovich J, Hargreave FE, Chalmers R, Shier KJ, Gauldie J, Bienenstock J. Late cutaneous allergic responses in isolated IgE-dependent reactions. J Allergy Clin Immunol 1973; 52:38–46.
33. Kirby JG, Robertson DG, Hargreave FE, Dolovich J. Asthmatic responses to inhalation of anti-human IgE. Clin Allergy 1986; 16:191–194.
34. Dorsch W, Baur X, Emslander HP, Fruhmann G. Zu Pathogenese und Therapie der allergeninduzierten verzögerten Bronchialobstruktion. Prax Pneumol 1980; 34: 461–468.
35. Cockcroft DW. Pharmacologic modulation of LPR: muscarinic cholinergic antagonists. In: Dorsch W, ed. Late Phase Allergic Reactions. Boca Raton, FL: CRC Press, 1990:277–281.
36. Boulet L-P, Roberts RS, Dolovich J, Hargreave FE. Prediction of late asthmatic responses to inhaled allergen. Clin Allergy 1984; 14:379–385.
37. Altounyan REC. Changes in histamine and atropine responsiveness as a guide to diagnosis and evaluation of therapy in obstructive airways disease. In: Pepys J, Franklands AW, eds. Disodium Cromoglycate in Allergic Airways Disease. London: Butterworths, 1970:47–53.
38. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in nonallergic bronchial reactivity. Clin Allergy 1977; 7:503–513.
39. Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. J Allergy Clin Immunol 1982; 70:170–177.
40. Boulet L-P, Cartier A, Thomson NC, Roberts RS, Dolovich J, Hargreave FE. Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. J Allergy Clin Immunol 1983; 71:399–406.
41. Cockcroft DW, Murdock KY. Changes in bronchial responsiveness to histamine at intervals after allergen challenge. Thorax 1987; 42:302–308.
42. Durham SR, Craddock CF, Cookson WO, Benson MK. Increases in airway responsiveness to histamine precede allergen-induced late asthmatic responses. J Allergy Clin Immunol 1988; 82:764–770.
43. Marsh WR, Irvin CG, Murphy KR, Behrens BL, Larsen GL. Increases in airway reactivity to histamine and inflammatory cells in bronchoalveolar lavage after the late asthmatic response in an animal model. Am Rev Respir Dis 1985; 131:875–879.
44. Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM. Bronchoalveolar cell profiles of asthmatic and non-asthmatic subjects. Am Rev Respir Dis 1987; 136:379–383.
45. Cockcroft DW. Allergen-induced increase in airway responsiveness: a model for examination of ''anti-inflammatory'' effects of asthma treatment. In: Morley J, ed. Preventive Therapy in Asthma. London: Academic Press, 1991:141–152.

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46. de Monchy JGR, Kauffman HF, Venge P, Koeter GH, Jansen HM, Sleuter HJ, de Vries K. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis 1985; 131:373–376.
47. Metzger WJ, Richerson HB, Wasserman SI. Generation and partial characterization of eosinophil chemotactic activity and neutrophil chemotactic activity during early and late phase asthmatic response. J Allergy Clin Immunol 1986; 78:282–290.
48. Pin I, Freitag AP, O'Byrne PM, Girgis-Gabardo A, Watson RM, Dolovich J, Denburg JA, Hargreave FE. Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. Am Rev Respir Dis 1992; 145:1265–1269.
49. Fabbri LM, Mapp CE, Hendrick DJ. Standardization of the dosimeter method for measurement of airway responsiveness in man. In: Hargreave FE, Woolcock AJ, eds. Airway Responsiveness: Measurement and Interpretation. Mississauga, Canada: Astra Pharmaceuticals Canada Ltd. 1985:29–34.
50. Sterk PJ, Fabbri LM, Quanjer PhH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo J-L. Airway responsiveness: standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Eur Respir J 1993; 6(16):53–83.
51. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. Clin Allergy 1977; 7:235–243.
52. Cockcroft DW, Swystun VA, Bhagat R. Interaction of inhaled β_2 agonist and inhaled corticosteroid on airway responsiveness to allergen and methacholine. Am J Respir Crit Care Med 1995; 152:1485–1489.
53. Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. Mechanisms of bronchial hyperreactivity in normal subjects after upper airway respiratory tract infection. Am Rev Respir Dis 1976; 113:131–139.
54. Lemanske RF, Dick EL, Swenson CA, Vrtis RF, Busse WW. Rhinovirus upper respiratory infection increases airway hyperreactivity and late asthmatic reactions. J Clin Invest 1989; 83:1–10.
55. Inman M, Watson R, Cockcroft DW, Wong, BJO, Hargreave FE, O'Byrne PM. Reproducibility of allergen-induced early and late asthmatic responses. J Allergy Clin Immunol 1995; 95:1191–1195.
56. Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR. Increases in activated T lymphocytes, eosinophils and cytokine mRNA expression for interleukin-5 and GM-CSF in bronchial biopsies after allergen challenge in atopic asthmatics. Am J Respir Cell Mol Biol 1993; 8:35–42.
57. Boulet LP, Chakir J, Dubé J, Laprise C, Boutet M, Laviolette M. Airway inflammation and structural changes in the development of airway hyperresponsiveness and symptomatic asthma: a review. Can Respir J 1998; 5:16–21.
58. Kay AB. Asthma and inflammation. J Allergy Clin Immunol 1991; 87:893–910.
59. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med 1998; 339:1194–1200.
60. Gomez E, Corrado OJ, Bladwin DL, Swanston AR, Favies RJ. Direct in vivo evidence of mast cell degranulation during allergen-induced reactions in man. J Allergy Clin Immunol 1986; 78:637–645.
61. Boulet, L-P. The influence of allergen exposure on asthma. Pract Allergy Immunol 1994; 9:17–22.

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62. Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. Am Rev Respir Dis 1989; 139:806–817.
63. Hendeles L, Harman E. Use of allergen bronchoprovocation to screen drugs for anti-asthma activity. Pharmacotherapy 1997; 17:39S–49S.
64. Boonsawat W, Salome CM, Woolcock AJ. Effect of allergen inhalation on the maximal response plateau of the dose-response curve to methacholine. Am Rev Respir Dis 1992; 146:565–569.
65. Boulet LP, Corbeil F, Turcotte H. Influence of a single antigenic challenge on the pattern of airway response to exercise in asthma. Ann Allergy 1992; 68:363–370.
66. Durham SR. The significance of late responses in asthma. Clin Exp Allergy 90; 21:3–7.
67. Frew AJ, Carroll MP, Gratziou C, Krug N. Endobronchial allergen challenge. Eur Respir J 1998; 11(suppl 26):33s–35s.
68. Tang C, Rolland JM, Ward C, Quan B, Walters EH. Allergen-induced airway reactions in atopic asthmatics correlate with allergen-specific IL-5 response by BAL cells. Respirology 1997; 2:45–55.
69. Gratziou C, Carroll M, Walls AF, Howarth PH, Holgate ST. Early changes in T-lymphocytes recovered by BAL following local allergen challenge of asthmatic airways. Am Rev Respir Dis 1992; 145:1259–1264.
70. Wenzel SE, Westcott JY, Larsen GL. Bronchoalveolar lavage fluid mediator levels 5 minutes after allergen challenge in atopic subjects with asthma: relationship to the development of late asthmatic responses. J Allergy Clin Immunol 1991; 87: 540–548.
71. Gratziou C, Carroll M, Montefort S, Teran L, Howarth PH, Holgate ST. Inflammatory and T-cell profile of asthmatic airways 6 hours after local allergen provocation. Am J Respir Crit Care Med 1996; 153:515–520.
72. Holgate ST, Bodey KS, Janezic A, Frew AJ, Kaplan AP, Teran LM. Release of RANTES, MIP-1 alpha, and MCP-1 into asthmatic airways following endobronchial allergen challenge. Am J Respir Crit Care Med 1997; 156:1377–1383.
73. Djukanovic R, Roche WR, Wilson JW, Beasley CRW, Twentyman OP, Howarth PH, et al. Mucosal inflammation in asthma: state of the art. Am Rev Respir Dis 1990; 142:434–457.
74. Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR. Increases in activated T lymphocytes, eosinophils, and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. Am J Respir Cell Mol Biol 1993; 8:35–42.
75. Aalbers R, de Monchy JG, Kauffman HF, Smith M, Hoekstra Y, Vrugt B, Timens W. Dynamics of eosinophil infiltration in the bronchial mucosa before and after the late asthmatic reaction. Eur Respir J 1993; 6:840–847.
76. Pizzichini MM, Popov TA, Efthimiadis A, Hussack P, Evans S, Pizzichini E, Dolovich J, Hargreave FE. Spontaneous and induced sputum to measure indices of airway inflammation in asthma. Am J Respir Crit Care Med 1996; 154:866–869.

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77. Byrne PM. Increases in airway eosinophils and interleukin-5 with minimal bronchoconstriction during repeated low-dose allergen challenge in atopic asthmatics. Eur Respir J 1998; 11:821–827.
78. Fahy JV, Liu J, Wong H, Boushey HA. Analysis of cellular and biochemical constituents of induced sputum after allergen challenge: a method for studying allergic airway inflammation. J Allergy Clin Immunol 1994; 93:1031–1039.
79. Sulakvelidze I, Inman MD, Rerecich T, O'Byrne PM. Increases in airway eosinophils and interleukin-5 with minimal bronchoconstriction during repeated low-dose allergen challenge in atopic asthmatics. Eur Respir J 1998; 11:821–827.
80. Palmqvist M, Pettersson K, Sjöstrand M, Andersson B, Löwhagen O, Lötvall J. Mild experimental exacerbation of asthma induced by individualized low-dose repeated allergen exposure. A double-blind evaluation. Respir Med 1998; 10:1223–1230.
81. Boulay ME, Bernier MC, Boulet LP. Effects of low-dose allergen challenge (LDAC) on lower airways in non-asthmatic subjects with allergic rhinitis (NAAR). Am J Resp Crit Care Med 2001; 163:A435.
82. Gizycki MJ, Adelroth E, Rogers AV, O'Byrne PM, Jeffery PK. Myofibroblast involvement in the allergen-induced late response in mild atopic asthma. Am J Respir Cell Mol Biol 1997; 6:664–673.
83. Becky Kelly EA, Busse WW, Jarjour NN. Increased matrix metalloproteinase-9 in the airway after allergen challenge. Am J Respir Crit Care Med 2000; 162:1157–1161.
84. Chakir J, Boulay ME, Deschesnes F, Boulet LP. Allergen challenge (AC) increases matrix metalloproteinase-9 (MMP-9) in the sputum of allergic asthmatic (AA) subjects. Am J Respir Crit Care Med 163(5):A789.
85. Boulet LP, Becker A, Berube D, Beveridge R, Ernst P. Canadian Asthma Consensus Report, 1999. Canadian Asthma Consensus Group. CMAJ 1999; 161(11 Suppl): S1–61.
86. International Consensus Report on Diagnosis and Treatment of Asthma. International Asthma Management Project. Allergy 1992; 47(13 Suppl):1–61.
87. Wong BJ, Dolovich J, Ramsdale EH, O'Byrne P, Gontovnick L, Denburg JA, Hargreave FE. Formoterol compared with beclomethasone and placebo on allergen-induced asthmatic responses. Am Rev Respir Dis 1992; 146:1158–1160.
88. Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate and beclomethasone dipropionate on allergen-induced early asthmatic responses, late asthmatic responses and increased bronchial responsiveness to histamine. J Allergy Clin Immunol 1987; 79:734–740.
89. Crescioli S, Spinazzi A, Plebani M, Pozzani M, Mapp CE, Boschetto P, Fabbri LM. Theophylline inhibits early and late asthmatic reactions induced by allergens in asthmatic subjects. Ann Allergy 1991; 66(3):245–251.
90. Crimi E, Violante B, Pellegrino R, Brusasco V. Effect of multiple doses of nedocromil sodium given after allergen inhalation in asthma. J Allergy Clin Immunol 1993; 92(6):777–783.
91. Hui KP, Taylor IK, Taylor GW, Rubin P, Kesterson J, Barnes NC, Barnes PJ. Effect of a 5-lipoxygenase inhibitor on leukotriene generation and airway responses after allergen challenge in asthmatic patients. Thorax 1991; 46(3):184–189.

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92. Bel EH, Timmers MC, Dijkman JH, Stahl EG, Sterk PJ. The effect of an inhaled leukotriene antagonist, L-648,051, on early and late asthmatic reactions and subsequent increase in airway responsiveness in man. J Allergy Clin Immunol 1990; 85(6):1067–1075.
93. De Baets FM, Goeteyn M, Kerrebijn KF. The effect of two months of treatment with inhaled budesonide on branchial responsiveness to histamine and house-dust mite antigen in asthmatic children. Am Rev Respir Dis 1990; 142:581–586.
94. Dahl R, Johansson S-A. Importance of duration of treatment with inhaled budesonide on the immediate and late bronchial reaction. Eur J Respir Dis 1982; 62 (suppl 122):167–175.
95. Swystun VA, Bhagat R, Kalra S, Jennings B, Cockcroft DW. Comparison of 3 different doses of budesonide and placebo on the early asthmatic response (EAR) to inhaled allergen. J Allergy Clin Immunol 1998; 102:363–367.
96. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. Lancet 1990; 336:1338–1342.
97. Booij-Noord H, Orie NGM, deVries K. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J Allergy Clin Immunol 1971; 48:344–354.
98. Aalbers R; Kauffman HF; Groen H; Koëter GH, de Monchy JG. The effect of nedocromil sodium on the early and late reaction and allergen-induced bronchial hyperresponsiveness. J Allergy Clin Immunol 1991; 87:993–1001.
99. Cockcroft DW, McParland CP, O'Byrne PM, Manning P, Friend JL, Rutherford BC, Swystun VA. Beclomethasone given after the early asthmatic response inhibits the late response and the increased methacholine responsiveness and cromolyn does not. J Allergy Clin Immunol 1993; 91:1163–1168.
100. Cockcroft DW, Murdock KY, Gore BP, O'Byrne PM, Manning P. Theophylline does not inhibit allergen-induced increase in airway responsiveness to methacholine. J Allergy Clin Immunol 1989; 83:913–920.
101. Cockcroft DW. Airway hyperresponsiveness and late asthmatic responses. Chest 1988; 94:178–180.
102. Boulet LP, Turcotte H, Boutet M, Dubé J, Gagnon M, Laviolette M. Influence of salmeterol on chronic and allergen-induced airway inflammation in mild allergic asthma: a pilot study. Curr Ther Res 1997; 58:240–259.
103. Boulet LP, Chakir J, Milot J, Boutet M, Laviolette M. Effect of salmeterol on allergen-induced airway inflammation in mild allergic asthma. Clin Exp Allergy 2001; 31:430–437.
104. Gauvreau GM, Watson RM, Jordana M, Cockcroft D, O'Byrne PM, The effect of regular inhaled salbutamol on allergen-induced airway responses and inflammatory cells in blood and induced sputum. Am J Respir Crit Care Med 1997; 156:1738–1745.
105. Calhoun WJ, Hinton KL, Kratzenberg JJ. The effect of salmeterol on markers of airway inflammation following segmental allergen challenge. Am J Respir Crit Care Med 2001; 163:881–886.
106. Boulet LP, Chapman KR, Côté J, Kalra S, Bhagat R, Swystun VA, Laviolette M, Cleland LD, Deschesnes F, Su JK, DeVault A, Fick RB, Cockcroft DW. Inhibitory

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effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. Am J Respir Crit Care Med 1997; 155:1835–1840.
107. Lechie MJ, ten Brinkle A, Lordan J, et al. 240563, a humanized anti-IL5 monoclonal antibody:initial single dose safety and activity in patients with asthma. Am J Respir Crit Care Med 1999; 159:A624.
108. Barnes PJ. Inhaled glucocorticoids for asthma. N Engl J Med 1995; 332:868–875.
109. Ernst P, Spitzer WO, Suissa S, Cockcroft D, Habbick B, Horwitz RI, Boivin JF, McNutt M, Buist AS. Risk of fatal and near-fatal asthma in relation to inhaled corticosteroid use. JAMA 1992; 268:3462–3464.
110. Rutten van Mölken MP, Van Doorslaer EK, Jansen MC, Kerstjens HA, Rutten FF. Costs and effects of inhaled corticosteroids and bronchodilators in asthma and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1995; 151: 975–982.
111. Hoppe M, Harman E, Hendeles L. The effect of inhaled gallopamil, a potent calcium channel blocker, on the late-phase response in subjects with allergic asthma. J Allergy Clin Immunol 1992; 89:688–695.
112. Cockcroft DW, Keshmiri M, Murdock KY, Gore BC. Allergen-induced increase in airway responsiveness is not inhibited by acute treatment with ketotifen or clemastine. Ann Allergy 1992; 68:245–250.
113. Boulet LP, Cockcroft DW, Toogood J, Lacasse Y, Baskerville J, Hargreave FE. Comparative assessment of safety and efficacy of inhaled corticosteroids: report of a committee of the Canadian Thoracic Society. Eur Respir J 1998; 11:1194–1210.
114. Marshik PL, Thomas D, Ahrens R, Braver H, Hendeles L. Dose-response of inhaled beclomethasone (B) for attenuating the airway responses to allergen. J Allergy Clin Immunol 1996; 97:316.
115. Kidney JC, Cockcroft DW, Hargreave FE, Boulet LP, Jennings B. Evaluation of inhaled corticosteroid activity using single doses and allergen challenge model. J Allergy Clin Immunol 1997; 100:65–70.
116. Laprise C, Boulet LP. Asymptomatic airway hyperresponsiveness: A three-year follow-up. Am J Respir Crit Care Med 1997; 156:403–409.
117. Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a β_2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind, parallel-group controlled trial. J Allergy Clin Immunol 1992; 90:32–42.
118. Boulet LP. Long-versus short-acting beta$_2$-agonists. Implications for asthma management. Drugs 1994; 47:207–222.
119. McIvor RA, Pizzichini E, Turner MO, Hussack P, Hargreave FE, Sears MR. Potential masking effects of salmeterol on airway inflammation in asthma. Am J Respir Crit Care Med 1998; 158:924–930.
120. Pizzichini MMM, Kidney JC, Wong BJO, Morris MM, Efthimiadis A, Dolovich J, Hargreave FE. Effect of salmeterol compared with beclomethasone on allergen-induced asthmatic and inflammatory responses. Eur Respir J 1996; 9:449–455.
121. Verdiani P, Di Carlo S, Baronti A, Bianco S. Effect of inhaled frusemide on the early response to antigen and subsequent change in airway reactivity in atopic patients. Thorax 1990; 45:377–381.

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10

Pathophysiology of the Airway Response to Inhaled Allergen in Asthmatic Subjects

Role of IgE

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I. Introduction

Subjects with allergic asthma who inhale an aeroallergen to which they are sensitive become symptomatic with wheeze, cough, chest tightness, and sputum production. Common aeroallergens that evoke this response include cat skin allergen (*Fel d* 1) and house dust mite allergens (*Der p* 1 and *Der p* 2). When airway allergen challenge is performed under carefully controlled conditions in the clinical research laboratory, pulmonary function tests reveal airway obstruction and worsening of bronchial hyperreactivity to histamine or methacholine. Specifically, asthmatic subjects typically demonstrate two patterns of response to inhaled allergen in this setting. Nearly all subjects develop an early asthmatic response (EAR), i.e., symptoms and bronchoconstriction commencing within minutes of inhalation of allergen inhalation and resolving spontaneously within 1 or 2 hours. In addition, approximately 50–60% of asthmatic subjects also develop a late asthmatic response (LAR), i.e., recurrence of symptoms of wheeze and recurrence of bronchoconstriction 3–12 hours after allergen challenge. Exploring the mechanisms of the airway response to inhaled allergen has helped in the development of current theories about the pathophysiology of asthma, and the allergen challenge model has also helped in the development process for new drugs for asthma.

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In this chapter I will review the pathophysiology of the early and late phase responses to inhaled allergen in asthmatic subjects. Special attention will be paid to the role of IgE and to the new insights that have been gained from clinical studies involving the effects of nonanaphylactogenic anti-IgE antibodies in allergen challenge experiments in animals and humans.

II. Mechanism of the Early Asthmatic Response

The bronchospasm that occurs immediately after aeroallergen inhalation in allergic asthmatic subjects is at least partly an IgE-mediated immediate hypersensitivity reaction (1). At least two lines of evidence support this mechanism for the early asthmatic response. First, pretreatment with an anti-IgE monoclonal antibody attenuates the early phase response in asthmatic subjects (Fig. 1) (2,3). Second, analysis of bronchoalveolar lavage fluid collected immediately after air-

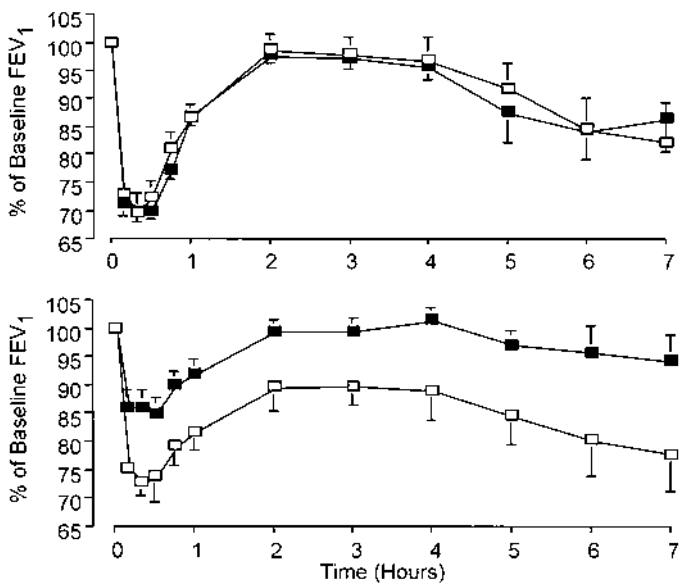

Figure 1 The effect of anti-IgE on the early and late phase response to inhaled allergen. The data are from the study presented in Figure 2. The figure shows the FEV_1 as a percent of baseline in the first hour after allergen challenge (early phase response) and from 2 to 7 hours after allergen challenge (late phase response) in the placebo (top panel)– and rhuMAb-E25 (lower panel)–treated groups at baseline (open squares) and at the end of treatment (closed squares). rhuMAb-E25 treatment significantly attenuated both the early and late phase responses.

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way allergen challenge reveals increased concentrations of mediators such as histamine, tryptase, PGD_2, thromboxane A_2 and B_2, and LTC_4 and D_4, which are known to be released by mast cells, a cell type that expresses a high density of receptors for IgE on its surface (4–8).

In immediate hypersensitivity reactions, multivalent allergen molecules crosslink adjacent Fab components of IgE on mast cells and basophils, causing degranulation of the cells and secretion of preformed and newly generated mediators of bronchoconstriction and vascular permeability. Specifically, aeroallergen associates with IgE bound to its high-affinity receptor (FcεRI) on the membrane of effector cells. The high-affinity receptor (FcεRI) is a multimeric complex consisting of one IgE-binding α chain, one β chain, and two disulfide linked γ chains (9); the α chain binds IgE, and the β and γ chains are required for insertion of the α chain into the membrane and for signal transduction. Signal transduction following receptor aggregation involves activation of the protein kinase Lyn, phosphorylation of tyrosine residues on the β and γ chains of the FcεR1 receptor, activation of tyrosine kinase Syk, phosphorylation of phospholipases, and degranulation of the cell granules via non cytoxic calcium- and temperature-dependent mechanisms (10).

The mediators released by mast cells following IgE-mediated activation include those that were preformed and stored in secretory granules and those that are newly generated (11). Preformed mediators include histamine, neutral proteases (tryptase and chymase), proteoglycans, and acid hydrolases (β-hexosaminidase and β-glucuronidase). Several mediators are also newly generated, and these include arachidonic acid metabolites, platelet-activating factor, and cytokines. Metabolites of arachidonic acid are released from cellular phospholipids upon activation of phospholipase enzymes. Biologically active arachidonic acid derivatives include the prostaglandin, leukotriene, and lipoxin families (Fig. 2). Free arachidonic acid is metabolized to prostanoids (PGD2, PGE2, PGF2α, prostacyclin, and thromboxane A_2) by the action of cyclooxygenase or to leukotrienes by the action of lipoxygenases. The 5-lipoxygenase pathway, in particular, is considered important because it yields LTA_4, LTC_4, LTD_4, and LTE_4 (12). Platelet-activating factor (PAF) is generated from alkyl phospholiids after cell activation and a variety of cytokines, including IL-3, IL-4, IL-5, IL-6, IFNγ, GM-CSF, and TNFα, are also newly synthesized and secreted by activated mast cells (11,13).

Many of the preformed and newly synthesized mediators released from activated mast cells cause smooth muscle contraction either directly or indirectly, and acute bronchoconstriction is considered the principal mechanism of airway obstruction during the early asthmatic reaction. For example, histamine, PGD_2, PGF2α, thromboxane B_2, LTC_4, and LTD_4 are thought to cause contraction of airway smooth muscle directly (14–17), whereas the contraction caused by tryptase and PAF is indirect (18–21). Although all of these mediators have the poten-

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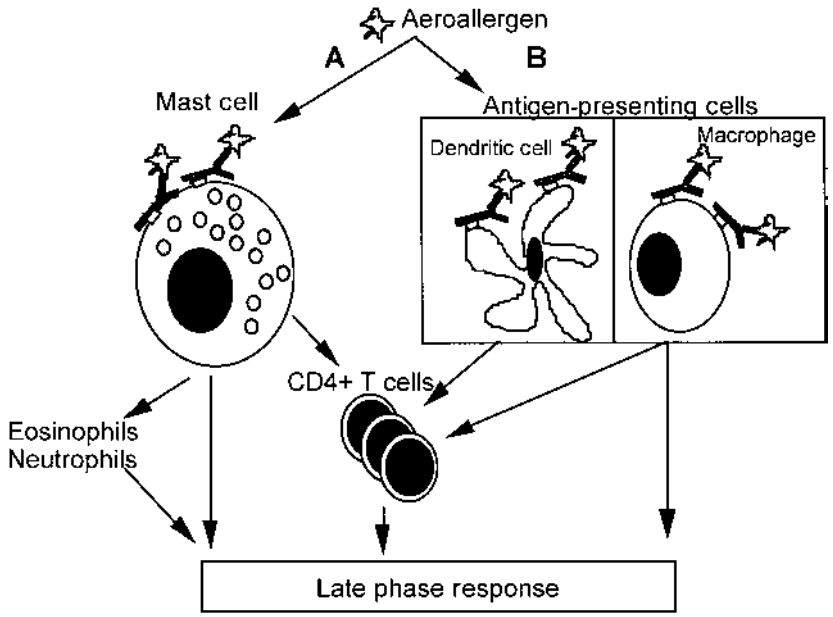

Figure 2 Schematic representation of possible IgE-mediated mechanisms for the late phase response involving mast cell–dependent and mast cell–independent pathways. Inhalation of aeroallergen may cause IgE-dependent activation of mast cells leading directly or indirectly to the late phase response (pathway A). Alternatively, there is evidence that inhalation of aeroallergen may cause activation of CD4+ T cells and a subsequent late phase response through a process of IgE-facilitated antigen presentation or IgE-dependent antigen focusing of allergen to CD4+ T cells by IgE receptor–bearing dendritic cells (pathway B).

tial to play some role in the bronchoconstriction of the early asthmatic response, several lines of evidence suggest that LTC_4 and LTD_4 are the most important mediators of this response. First, the leukotrienes are the most potent smooth muscle agonists on a molar basis, being at least one order of magnitude more potent than histamine or cholinergic agonists (22). Second, pretreatment of asthmatic subjects with cysteinyl leukotriene receptor antagonists is associated with a near complete attenuation of the early phase response to aerosolized allergen challenge (23,24). Third, antagonists of other mast cell mediators such as histamine, cycloxygenase products, and PAF are only marginally effective in attenuating the EAR, if effective at all. For example, the inhibitory effects of antihistamines on the early asthmatic response are modest (25–27), clinical trials of at least three different PAF antagonists have not shown efficacy in blocking the

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EAR (28–30), and indomethacin (an inhibitor of cyclooxygenase) does not attenuate the EAR (31).

Increased vascular permeability and mucin hypersecretion represent other possible mechanisms by which mast cell mediators might cause airway obstruction during the EAR. Indeed, albumin concentrations in bronchoalveolar lavage increase immediately following allergen challenge (32) providing evidence of allergen-induced increases in vascular permeability. These effects are likely to be mediated by many of the same mediators that cause smooth muscle contraction, including leukotrienes and histamine; chymase may have a special role as a mucin secretagogue (33). The airway narrowing during the EAR appears to be mainly the result of bronchial smooth muscle contraction, however, because the airway narrowing is reversible with β-agonist treatment (34), which would not be expected to reverse mucosal edema or mucin secretion.

It is possible that the mechanism of bronchoconstriction during the EAR is not entirely IgE dependent. In fact, anti-IgE pretreatment does not completely block bronchoconstriction during the EAR in human studies (2,35). Although the size or duration of dosing with anti-IgE in these protocols may have been inadequate, it is possible that non–IgE-dependent mechanisms may operate during the EAR. The non-IgE mechanisms that may cause mast cell degranulation include complement-derived anaphylatoxins, especially C3a (36), or nonspecific stimuli such as hyperosmolarity (37). In addition, it is possible that the rate of transepithelial electrolyte transport by airway epithelial cells is important in the development of the EAR. This possibility is suggested by the remarkable efficacy of inhaled furosemide, an inhibitor of Na-K-Cl cotransport channels, in inhibiting the EAR (38). One possibility to explain this finding is that mast cell mediators such as arachidonic acid metabolites cause increased transepithelial electrolyte transport and that this is an important final common pathway for the EAR. Whether or not changes in electrolyte transport during allergen challenge are IgE dependent is not known.

In summary, current information suggests that the mechanism of airway narrowing during the early asthmatic response is an acute bronchoconstrictor response caused mainly by an IgE-dependent immediate hypersensitivity reaction. Of the preformed and newly synthesized mediators released from mast cells during these reactions, the cysteinyl leukotrienes appear to be the most important in the pathogenesis of the EAR.

III. Mechanism of the Late Asthmatic Response

A. Overview

Approximately 50–60% of patients who develop an EAR to allergen also develop delayed bronchoconstriction, otherwise known as a late asthmatic response (39).

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The physiological events that characterize the LAR include not only recurrent bronchoconstriction but also worsening of baseline hyperresponsiveness to methacholine or histamine (39). Pathologically, the LAR is characterized by cellular inflammation of the airway, increased bronchovascular permeability, and increased mucus secretion (40–42). Together, these findings suggest that the airway obstruction characteristic of the LAR results from a combination of effects including smooth muscle contraction, airway edema, and mucus hypersecretion. The dominant cause of airway obstruction during the LAR must be bronchial smooth muscle contraction, however, because, like the EAR, the obstruction is reversible with treatment with β-agonist (43,44). The characteristics of asthmatic subjects who develop both early and late asthmatic responses to aeroallergen are incompletely understood. So-called ''dual phase'' responders are not consistently found to have more severe asthma than ''single phase'' responders, but they are more reactive to methacholine at baseline (45).

The mechanisms responsible for the delayed bronchoconstriction, mucosal edema, and mucus hypersecretion characteristic of the LAR are incompletely understood, but the association of LAR with increased airway inflammation is well established. For example, analysis of airway secretions and of airway biopsies during the LAR demonstrates increased numbers of eosinophils, lymphocytes (especially T lymphocytes of the Th2 phenotype), and basophils (46–51). The increase in neutrophil numbers sometimes observed during the LAR may be a nonspecific phenomenon (52). In addition, increases in the inflammatory products of eosinophils and lymphocytes have been described in the airway during the LAR (46,53).

The recruitment of eosinophils, lymphocytes, and other inflammatory cells from the vascular space to the airways during the LAR is delayed because recruitment requires the coordinated upregulation of a number of gene products related to transmigration of cells (54–56). First, a series of receptor-ligand interactions must occur between leukocytes and vascular endothelial cells during the initiation of transmigration. Selectins, expressed on leukocytes, bind to their ligands on endothelial cells to initiate tethering and rolling, the first step in endothelial transmigration (56). During the LAR, mast cell–derived TNFα and GM-CSF may be important in upregulating selectins and their ligands (46). In addition, epithelial cells activated during the EAR may also secrete mediators such as IL-6 that can upregulate selectins and their ligands. Tethering and rolling is followed by firm adhesion mediated by integrins, a family of adhesion molecules that is known to be upregulated by cytokines such as IL-4 and IL-13 (57–59). Integrin $\alpha_4\beta_1$ (formerly very late activation molecule 4, VLA-4) interacts with its receptor on vascular endothelial cells, CD106 (formerly vascular cell adhesion molecule 1, VCAM-1) to promote firm adhesion of eosinophils and lymphocytes. Chemotaxis is the final step in inflammatory cell recruitment, and chemoattractants, such as IL-5, PAF, RANTES, PGD_2, LTD_4, and eotaxin, may be particularly relevant for

the eosinophilia observed during the LAR. These chemoattractants may be secreted by mast cells, epithelial cells, or macrophages activated directly or indirectly by allergen exposure; they bind to matrix molecules in the interstitium, creating a gradient along which inflammatory cells migrate to the airway interstitium and epithelium from the vascular space.

B. Role of IgE in the Late Phase Response to Allergen

The role of IgE in the late asthmatic response has been established in human studies in which it has been shown that pretreatment of asthmatic subjects with rhuMAb-E25—a nonanaphylactogenic anti-IgE monoclonal antibody—attenuates the LAR (Fig. 1) (35). This study established a role for IgE in the LAR—a role that had been debated up to that point (60). It also provided suggestive evidence that anti-IgE treatment attenuates allergen-induced airway eosinophilia.

The precise mechanisms by which IgE contributes to the inflammatory events of the LAR are not fully worked out. Because IgE can bind to effector cells via either high-affinity receptors (FcεRI) or low-affinity receptors (FcεRII/CD23), it is possible that IgE mediates the LAR as a consequence of its binding to FcεRI receptors, FcεRII receptors, or both. A review of the literature on this subject suggests at least two possible mechanisms for IgE-mediated airway narrowing and hyperreactivity during the LAR. One mechanism depends on IgE and mast cells; a second mechanism depends on IgE but does not require mast cells.

C. IgE-Dependent, Mast Cell–Dependent Mechanism for the LAR

Mast cells are among the most important and potent effector cells for IgE-mediated inflammatory responses. An IgE/mast cell–dependent mechanism for bronchoconstriction during the LAR is plausible, because mast cells are equipped with the necessary mediators to cause delayed bronchoconstriction following aeroallergen exposure and crosslinking of surface FcεRI receptors. A variety of mast cell mediators may be important in causing the delayed bronchoconstriction typical of the LAR, but IL-5 may be particularly important. IL-5 has been identified as a product of activated mast cells (61), and IL-5 is an important chemoattractant for eosinophils. Thus, accumulation and activation of eosinophils in the airway following mast cell activation during the EAR might account for bronchoconstriction during the LAR secondary to the effects of eosinophil mediators such as MBP, ECP, and leukotrienes.

Secretion of eosinophil-active cytokines such as IL-5 is not the only mechanism by which mast cells may mediate the LAR. Other cytokines such as IL-4 are also secreted by mast cells (62), and IL-4 might indirectly mediate the LAR through its upregulatory effects on T cells or epithelial cells (63–65). In addition, other mast cell mediators such as arachidonic acid metabolites may cause activation of airway epithelial cells or macrophages, setting off a cascade of events

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that could culminate in the LAR. Thus, the IgE-dependent activation of mast cells may mediate the LAR via the effects of mast cells on eosinophil chemotaxis, epithelial cell activation, macrophage activation, or other indirect effects in the airway.

The role of IL-5 in the late phase response to allergen has been explored in animal studies, and some preliminary data are also available from a small study in human subjects. For example, Cieslewicz et al. (66) recently demonstrated that pretreatment of sensitized mice with an antibody to IL-5 prevents increases in airway resistance and airway eosinophilia 3–8 hours following airway challenge with allergen. Studies of the effects of IL-5 antibodies are ongoing in allergic asthmatic subjects as well, and preliminary data have been presented (67). Twelve asthmatic subjects were treated with a single injection of anti-IL-5, which resulted in a significant decline in blood and sputum eosinophilia. Despite the antieosinophil effects of the anti-IL-5 treatment, no significant attenuation of the early or late asthmatic response was found. Although this study calls into question the relevance of IL-5 and eosinophilia as mediators of the LAR in humans, the authors cautioned that their study was small and may not have used an adequate dose of anti-IL-5. Additional human studies with anti-IL-5 should clarify this issue.

D. IgE-Dependent, Mast Cell–Independent Mechanism for the LAR

Significant advances have been made in the past 15 years in understanding the role of IgE and its receptors in allergic inflammation. A particularly important advance has been the discovery that IgE and its receptors have a role in antigen presentation (68,69). Specifically, it has been discovered that FcεRI and FcεRII/CD23 are present on the cell surface of antigen-presenting cells (APCs) such as B cells and dendritic cells (70–72). Interestingly, supernatants of activated Th2 cells strongly enhance expression of FceRII/CD23 on the cell surface of APCs in vitro (73), explained predominantly as another effect of the Th-2–derived cytokine IL-4 (68,74,75). The presence of IgE receptors on APCs means that these cells can bind allergen-specific IgE by an FcεR mechanism, and this mechanism greatly increases the efficiency of antigen presentation, as evidenced by the experiments of van der Heijden et al. (68). These authors examined the role of IgE in *Der p* II antigen presentation by CD23-expressing EBV-B cells to *Der p* II–specific CD4+ T lymphocytes. Purified *Der p* II protein was preincubated with IgE from atopic donors, and the EBV-B cells were incubated with these complexes before the B cells were used as APCs. It was found that activation of the *Der p* II–specific T cells by the IgE-complexed *Der p* II occurred at a 1000-fold lower concentration than that required for T-cell activation by presentation of uncomplexed *Der p* II. This role of IgE in antigen presentation to APCs has been termed ''facilitated antigen presentation'' (68) or ''IgE-dependent antigen

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focusing'' (70). The finding of IgE receptors on the surface of antigen-presenting cells in the skin has shown that this mechanism might be operant in human allergy (71).

The discovery of the phenomenon of IgE-dependent antigen focusing has opened up the possibility of an IgE-dependent, mast cell–independent, mechanism for the LAR. In this scenario mast cells are not required for the development of a late asthmatic response. Rather, the important cells are IgE-bearing antigen-presenting cells, the most important of which are dendritic cells and macrophages. These IgE-bearing APCs bind inhaled allergen and present it to allergen-specific T cells, which then become activated and secrete cytokines of the Th2 phenotype, resulting in eosinophil recruitment and bronchoconstriction (Fig. 2).

Evidence of an IgE-dependent, mast cell–independent, mechanism for the LAR comes mainly from the work of Coyle et al. (76) in the mouse model of asthma. These authors examined the role of IgE, IgE receptors, and mast cells in allergen-induced eosinophilia (a marker of the LAR) and T-cell activation in the lungs in sensitized mice. They found that administration of anti-IgE 6 hours before airway challenge with house dust mite antigen neutralized serum IgE, inhibited allergen-induced recruitment of eosinophils, and inhibited activation of T cells. They also found that mice deficient in CD23 and mice pretreated with an antibody to CD23 did not develop lung eosinophilia following allergen challenge. In contrast, mice deficient in mast cells continued to develop lung eosinophilia when challenged with allergen. From these data the authors concluded that the development of lung eosinophilia in response to allergen challenge does not depend on activation of mast cells, but rather on activation of T cells following IgE-CD23-facilitated antigen presentation to T cells. Dendritic cells in mice differ from those in humans in that they do not have FcεRI receptors, so any similar mechanism in humans may involve either high- or low-affinity IgE receptors.

E. IgE-Independent Mechanism for the LAR

While treatment with anti-IgE monoclonal antibody attenuated the LAR to allergen challenge in asthmatic human subjects, it did not abolish it (Fig. 1) (3). Again, the size or duration of dosing with anti-IgE in this protocol may simply have been inadequate, but it is possible that non–IgE-dependent mechanisms may contribute to the LAR. The evidence for this comes largely from studies of murine models of asthma, in which the combination of an increase in bronchial reactivity to methacholine and an increase in the number of eosinophils in bronchial lavage fluid within 24 hours after antigen challenge is thought to indicate the occurrence of events similar to those of the LAR in humans. These changes have been found after antigen challenge in mice incapable of synthesizing IgE or any other immune globulin (77). Further work in mice has suggested that antigen-induced lymphocyte synthesis of IL-13 may be sufficient to initiate the events responsible

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for increased bronchial reactivity and airway eosinophilia (78,79). The relevance of these findings to asthma as it occurs in humans is not established, but they at least raise the possibility that IgE serves as an important mechanism for amplifying the airway responses to antigen challenge but may not be entirely responsible for them.

IV. Summary and Conclusions

The mechanism of the LAR in allergic asthmatic subjects is incompletely understood, but an important role for IgE has been established. Whether IgE acts via mast cell–dependent or–independent mechanisms and whether IgE is fundamental to the occurrence of the LAR or instead amplifies the effects of other, non–IgE-dependent mechanisms in shaping the LAR is a source of ongoing debate and investigation.

The availability of nonanaphylactogenic anti-IgE antibodies for use in human studies has allowed clinical studies, which have established a role for IgE in the LAR. This finding also raises the possibility that IgE-dependent antigen focusing is important in allergic responses in the airway. Therefore, inhibition of IgE might represent a method for desensitizing the airway to aeroallergen and for interrupting an important mechanism of chronic allergic airway inflammation.

References

1. Holgate ST, Robinson C, Church MK. Mediators of immediate hypersensitivity. In: Middleston E, Reed CE, Regis EF, Adkinson NF, Yuninger JW, Busse WW, eds. Allergy Principles and Practice. 4th Ed. St. Louis: Mosby, 1993:267–301.
2. Boulet L-P, Chapman KR, Cote J, et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. Am J Respir Crit Care Med 1997; 155:1835–1840.
3. Fahy JV, Cockcroft DW, Boulet LP, et al. Effect of aerosolized anti-IgE (E25) on airway responses to inhaled allergen in asthmatic subjects. Am J Respir Crit Care Med 1998; 157:A410.
4. Casale TB, Wood D, Richerson HB, Zehr B, Zavala D, Hunninghake GW. Direct evidence of a role for mast cells in the pathogenesis of antigen-induced bronchoconstriction. J Clin Invest 1987; 80:1507–1511.
5. Wenzel SE, Fowler AA, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge: in vivo release of histamine and tryptase in atopic subjects with and without asthma. Am Rev Respir Dis 1988; 137:1002–1008.
6. Wenzel SE, Larsen GL, Johnston K, Voelkel NF, Westcott JY. Elevated levels of leukotriene C4 in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. Am Rev Respir Dis 1990; 142:112–119.
7. Wenzel SE, Westcott JY, Larsen GL. Bronchoalveolar lavage fluid mediator levels 5 minutes after allergen challenge in atopic subjects with asthma: relationship to the

Marcel Dekker, Inc.
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development of late asthmatic responses. J Allergy Clin Immunol 1991; 87:540–548.
8. Liu MC, Hubbard WC, Proud D, et al. Immediate and late inflammatory responses to ragweed antigen challenge of the peripheral airways in allergic asthmatics. Am Rev Respir Dis 1991; 144:51–58.
9. Bonnefoy J-Y, Aubry J-P, Gauchat J-F, et al. Receptors for lgE. Curr Opin Immunol 1993; 5:944–949.
10. Siraganian RP. Biochemical events in basophil or mast cell activation and mediator release. In: Middleton E, Reed CE, Ellis EF, Adkinson F, Yuninger JW, Busse WW, eds. Allergy Principles and Practice. Vol. 1. St. Louis: Mosby, 1998:204–227.
11. Church MK, Holgate ST, Shute JK, Walls AF, Sampson AP. Mast Cell-Derived mediators. In: Middleton E, Reed CE, Ellis EF, Adkinson NF, Yuninger JW, Busse WW, eds. Allergy Principles and Practices. Vol. 1. St. Louis: Mosby-Year Book, Inc., 1998:146–167.
12. Henderson WR. Role of leukotrienes in asthma. Ann Allergy 1994; 72:272–278.
13. Wasserman SI. Mast cells and airway inflammation in asthma. Am J Respir Crit Care Med 1994; 150:S39–S41.
14. Daniel EE, Triggle DJ. Structure and function of airway smooth muscle. In: Middleton E, Reed CE, Ellis EF, Adkinson NF, Yuninger JW, Busse WW, eds. Allergy Principles and Practices. Vol. 1. St. Louis: Mosby-Year Book, Inc., 1996:495–507.
15. Dahlen S-E, Hedqvist P, Hammarstrom S, Samuelsson B. Leukotrienes are potent constrictors of human bronchi. Science 1980; 288:484–486.
16. Svensson J, Stanberg K, Tuvemo T, Hamberd M. Effects on airway and vascular smooth muscle. Prostaglandins 1977; 14:245–436.
17. Drazen JM, Austen KF. Effects of intravenous administration of slow-reacting substance of anaphylaxis, histamine, bradykinin, and prostaglandin F 2α on pulmonary mechanics in the guinea pig. Journal of Clinical Investigation 1974; 53:1679–1685.
18. Johnson PRA, Armour CLA, Black JL. The action of platelet activating factor and its antagonism by WEB 2086 on human isolated airways. Eur Respir J 1990; 3:55–60.
19. Smith LJ, Rubin A-HE, Patterson R. Mechanism of platelet activating factor-induced bronchoconstriction. Am Rev Respir Dis 1988; 137:1015–1019.
20. Cuss FM, Dixon CMS, Barnes PJ. Effects of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. Lancet 1986; ii:189–192.
21. Johnson PR, Ammit AJ, Carlin SM, Armour CL, Caughey GH, Black JL. Mast cell tryptase potentiates histamine-induced contraction in human sensitized bronchus. Eur Respir J 1997; 10:38–43.
22. Barnes NC, Piper PJ, Costello JF. Comparative effects of inhaled leukotriene C4, leukotriene D4, and histamine in normal human subjects. Thorax 1984; 39:500–504.
23. Taylor IK, O'Shaughnessy KM, Fuller RW, Dollery CT. Effect of cysteinyl-leukotriene receptor antagonist ICI 204.219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. Lancet 1991; 337:690–694.
24. Diamant Z, Grootendorst DC, Veselic-Charvat M, et al. The effect of montelukast (MK-0476), a cysteinyl leukotriene receptor antagonist, on allergen-induced airway responses and sputum cell counts in asthma. Clin Exp Allergy 1999; 29:42–51.

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270 Madison Avenue, New York, New York 10016

25. Rafferty P, Beasley R, Holgate ST. The contribution of histamine to immediate bronchoconstriction provoked by inhaled allergen and adenosine 5′ monophosphate in atopic asthma. Am Rev Respir Dis 1987; 136:369–373.
26. de Bruin-Weller M, Rijssenbeek-Nouwens HM, de Monchy JGR. Lack of effect of cetirizine on early and late asthmatic responses after allergen challenge. J Allergy Clin Immunol 1994; 94:231–239.
27. Naclerio RM. The effect of antihistamines on the immediate allergic response: a comparative review. Otolaryngol Head Neck Surg 1993; 108:723–730.
28. Freitag A, Watson RM, Matsos G, Eastwood C, O'Byrne PM. Effect of a platelet activating factor antagonist WEB 2086, on allergen-induced asthmatic responses. Thorax 1993; 48:594–598.
29. Kuitert LM, Hui KP, Uthayarkumar S, et al. Effect of the platelet-activating factor antagonist UK-74,505 on the early and late response to allergen. Am Rev Respir Dis 1993; 147:82–86.
30. Evans DJ, Barnes PJ, Cluzel M, O'Connor BJ. Effects of a potent platelet-activating factor antagonist, SR27417A, on allergen-induced asthmatic responses. Am J Respir Crit Care Med 1997; 156:11–16.
31. Kirby JG, Hargreave FE, Cockcroft DW, O'Byrne PM. Effect of indomethacin o allergen-induced asthmatic responses. J Appl Physiol 1989; 66:578–583.
32. Fick RB, Metzger WJ, Richerson HB, et al. Increased bronchovascular permeability after allergen exposure in sensitive asthmatics. J Appl Physiol 1987; 63:1147–1155.
33. Sommerhoff CP, Caughey GH, Finkbeiner WE, Lazarus SCL, Basbaum CB, Nadel JA. Mast cell chymase; a potent secretagogue for airway gland serous cells. J Immunol 1989; 142:2450–2456.
34. Pepys J, Hutchkroft BJ. Bronchial challenge tests in etiologic diagnosis and analysis of asthma. Am Rev Respir Dis 1975; 112:829–859.
35. Fahy JV, Fleming HE, Wong HH, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med 1997; 155:1828–1834.
36. Mousli M, Hugli TE, Landry Y, Bronner C. A mechanism of action for anaphylatoxin C3a stimulation of mast cells. J Immunol 1992; 148:2456–2461.
37. Eggleston PA, Kagey-Sobokta A, Schleimer RP, Lichtenstein LM. Interaction between hyerosmolar and IgE-mediated histamine release from basophils and mast cells. Am Rev Respir Dis 1984; 130:86–91.
38. Bianco S, Pieroni MG, Refini RM, Rottoli L, Sestini P. Protective effect of inhaled furosemide on allergen-induced early and late asthmatic responses. N Engl J Med 1989; 321:1069–1073.
39. O'Byrne PM, Dolovich J, Hargreave FE. State of art: Late asthmatic responses. Am Rev Respir Dis 1987; 136:740–751.
40. Hargreave FE. Late phase asthmatic responses and airway inflammation. J Allergy Clin Immunol 1989; 83:525–527.
41. Dolovich J, Hargreave FE, Jordana M, Denburg J. Late phase airway reaction and inflammation. J Allergy Clin Immunol 1989; 83:521–524.
42. Tabachnik E, Schuster A, Gold WM, Nadel JA. Role of neutrophil elastase in allergen-induced lysozyme secretion in the dog trachea. J Appl Physiol 1992; 73:695–700.

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270 Madison Avenue, New York, New York 10016

43. Twentyman OP, Holgate ST. Reversibility of the allergen-provoked late asthmatic response by an inhaled β2-adrenoceptor agonist. Clin Exp Allergy 1994; 24:245–249.
44. Peebles RS, Permutt S, Togias A. Reversibility of the allergen-induced pulmonary late-phase reaction by an intravenous beta (2) agonist. J Appl Physiol 1998; 84: 1500–1505.
45. Avila PC, Segal M, Wong HH, Boushey HA, Fahy JV. Predictor of late asthmatic response; classification tree and logistic regression analyses. Am J Respir Crit Care Med 2000; 161:2092–2095.
46. Wooley KL, Adelroth E, Wooley MJ, Ellis R, Jordana M, O'Byrne PM. Effects of allergen challenge on eosinophils, eosinophil cationic protein, and granulocyte-macrophage colony stimulating factor in mild asthma. Am J Respir Crit Care Med 1995; 151:1915–1924.
47. Guo C-B, Liu MC, Galli SJ, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Identification of IgE-bearing cells in the late-phase response to antigen in the lung as basophils. Am J Respir Cell Mol Biol 1994; 10:384–390.
48. Fahy JV, Liu J, Wong H, Boushey HA. Cellular and biochemical analysis of induced sputum before and after allergen challenge of allergic asthmatic subjects. Am Rev Respir Dis 1993; 147:A551.
49. Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. Am Rev Respir Dis 1989; 139:806–817.
50. Gratziou C, Carroll M, Montefort S, Teran L, Howarth PH, Holgate ST. Inflammatory and T-cell profile of asthmatic airways 6 hours after local allergen provocation. Am J Respir Crit Care Med 1996; 153:515–520.
51. Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. J Allergy Clin Immunol 1993; 92:313–324.
52. Teran LM, Carroll MP, Frew AJ, Redington AE. Leukocyte recruitment after local endobronchial allergen challenge in asthma—relationship to procedure and to airway interleukin-8 release. Am J Respir Crit Care Med 1996; 154:469–476.
53. Kroegel C, Julius P, Matthys H, Virchow JC, Luttmann W. Endobronchial secretion of interleukin-13 following local allergen challenge in atopic asthma: relationship to interleukin-4 and eosinophil counts. Eur Respir J 1996; 9:899–904.
54. Albelda SM. Endothelial and epithelial cell adhesion molecules. Am J Respir Cell Mol Biol 1991; 4:195–203.
55. Albelda SM, Smith CW, Ward PA. Adhesion molecules and inflammatory injury. FASEB J 1994; 8:504–512.
56. Kansas GS. Selectins and their ligands: current concepts and controversies. Blood 1996; 88:3259–3287.
57. Mantovani A, Bussolino F, Dejana E. Cytokine regulation of endothelial cell function. FASEB J 1992; 6:2591–2598.
58. Abraham WM, Sielczak MW, Ahmed A, et al. α4-Integrins mediate antigen-induced late bronchial responses and prolonged airway hyperresponsiveness in sheep. J Clin Invest 1994; 93:776–787.

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270 Madison Avenue, New York, New York 10016

59. Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. J Immunol 1995; 154:799–803.
60. Zweiman B. The late phase response: role of IgE, its receptor, and cytokines. Curr Opin Immunol 1993; 5:950–955.
61. Bradding P, Roberts JA, Britten KM, et al. Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. Am J Respir Cell Mol Biol 1994; 10:471–480.
62. Bradding P, Feather IH, Howarth PH, et al. Interleukin 4 is localized to and released by human mast cells. J Exp Med 1992; 176:1381–1386.
63. DeKruyff RH, Turner T, Abrams JS, Palladino MA, Jr., Umetsu DT. Induction of human IgE synthesis by CD4+ T cell clones. Requirement for interleukin 4 and low molecular weight B cell growth factor. J Exp Med 1989; 170:1477–1493.
64. Paul WE. Interleukin 4: signalling mechanisms and control of T cell differentiation. Ciba Found Symp 1997; 204:208–216; discussion 216–219.
65. Bergmann M, Barnes PJ, Newton R. Molecular regulation of granulocyte macrophage colony-stimulating factor by human lung epithelial cells by interleukin (IL)-1 beta, IL-4, and IL-13 involves both transcriptional and posttranscriptional mechanisms. Am J Respir Cell Mol Biol 2000; 22:582–589.
66. Cieslewicz G, Tomkinson A, Adler A, et al. The late, but not early, asthmatic response is dependent on IL-5 and correlates with eosinophil infiltration. J Clin Invest 1999; 104:301–308.
67. Leckie MJ, ten Brinke A, Lordan J, et al. A humanized anti-IL-5 monoclonal antibody: initial single dose safety and activity in patients with asthma. Am J Respir Crit Care Med 1999:A624.
68. van der Heijden FL, van Neerven RJJ, van Katwijk M, Bos JD, Kapsenberg ML. Serum facilitated allergen presentation in atopic disease. J Immunol 1993; 150: 3643–3650.
69. Mudde GC, Bheeka R, Bruijnzeel-Koomen CAFM. IgE-mediated allergen presentation. Allergy 1995; 50:193–199.
70. Kehry MR, Yamashita LC. Low affinity IgE receptor (CD23) function on mouse B cells: role in IgE dependent antigen focusing. Proc Natl Acad Sci USA 1989; 86: 7556–7560.
71. Bieber T, De La Salle H, Wollenberg A, et al. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (FcεR1). J Exp Med 1992; 175:1285–1290.
72. Wang B, Rieger A, Kilgus O. Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεR1. J Exp Med 1992; 175:1353–1365.
73. van der Heijden FL, Wierenga EA, Bos JD, Kapsenberg ML. High frequency of IL-4-producing CD4+ allergen-specific T lymphocytes in atopic dermatitis lesional skin. J Invest Dermatol 1991; 97:389–394.
74. Vercelli D, Jabara HH, Lee B, Woodland N, Geha RS, Leung DYM. Human recombinant interleukin 4 induced FcεR2/CD23 on normal human monocytes. J Exp Med 1988; 167:1406.
75. Bieber T, Rieger A, Neuchrist C, et al. Induction of FcεR2/CD23 on human epidermal Langerhans cells by human recombinant interleukin 4 and γ interferon. J Exp Med 1989; 170:309–314.

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76. Coyle AJ, Wagner K, Bertrand C, Tsuyuki S, Bews J, Heusser C. Central role of immunoglobulin (lg) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. J Exp Med 1996; 183:1303–1310.
77. Corry DB, Grunig G, Hadeiba H, et al. Requirements for allergen-induced airway hyperreactivity in T and B cell-deficient mice. Mol Med 1998; 4:344–355.
78. Grunig G, Warnock M, Wakil AE, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. Science 1998; 282:2261–2263.
79. Wills-Karp M, Luyimbazi J, Xu X, et al. Interleukin-13: central mediator of allergic asthma. Science 1998; 282:2258–2261.

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11

Efficacy and Safety of Xolair Anti-IgE Monoclonal Antibody in Adult Asthmatics

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I. Introduction

The history of effective attempts to understand, treat, and manage asthma may be condensed into the past 40 years. By the mid-1960s asthma was recognized to have a significant allergic component even though the precise triggers of the disease were poorly characterized. IgE was described in 1967 reports by Ishizaka et al. (1) and Johansson and coworkers (2). Antecedent work had already demonstrated a role in the disease for basophil leukocytes (3). At the beginning of the 1980s short-term cell culture experiments incriminated specific mediators and the underlying involvement of the immune system. It was reasoned that when cell-bound IgE on basophils or tissue mast cells is crosslinked by an antigen, the cell degranulates, releasing preformed and newly synthesized mediators of immediate hypersensitivity such as histamine, leukotrienes, and platelet-activating factor. It was postulated that these pro-inflammatory chemicals identified in biological fluids might be responsible for the swelling, erythema and smooth muscle contraction characteristic of the asthmatic reaction.

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These in vitro and ex vivo data, coupled with emerging epidemiological data demonstrating a significant association between serum IgE levels and asthmatic airway disease, made the case for IgE as a key mediator in asthma. Elevations of IgE antibodies, not IgG, were reported by Pollart et al. (4) to be a risk factor for emergency department visits. In a population-based study of 2657 residents of Tucson, Arizona, self-reported symptoms of asthma correlated with serum IgE levels (5). Other studies confirmed these initial associations showing a relationship between IgE levels and airway hyperresponsiveness (6,7). Similar correlations between serum IgE and asthma have been strengthened by independent analyses of data from the U.S. National Health and Nutrition Surveys (8,9). These U.S. population–based studies demonstrated that the overall proportion of allergic (IgE-mediated) asthma among U.S. asthmatics ≥6 years old was 92% (9). Earlier NHANES II data revealed that 20.2% of U.S. participants had skin test reactivity to at least one of eight common perennial or seasonal allergens (8).

The availability in the mid-1990s of a nonanaphylactogenic antibody-binding IgE (10,11) made possible direct study of the precise role of IgE in clinical asthma. Boulet et al. (12), in an early use of this IgE binding MAb, determined the provocation concentration of allergen causing a 15% fall in FEV_1 (allergen PC15) during the early asthmatic response (EAR) employing an allergen-induced bronchoprovocation technique. Mean serum free IgE fell by 89% after anti-IgE (Xolair), while there was no significant change after placebo. Compared with baseline values (day 1), the median allergen PC15 on days 27, 55, and 77 were increased by 2.3, 2.2, and 2.7 doubling doses, respectively, with Xolair and −0.3, +0.1, and −0.8 doubling doses with placebo ($p < 0.002$). Methacholine PC20 improved after Xolair, this change becoming statistically significant on day 76 ($p < 0.05$); no change was observed in the placebo group. The role of IgE in early and late asthmatic reactions was also studied by Fahy and coworkers (13). Applying a similar aeroallergen bronchoprovocation technique to the study of 19 mild asthmatics, it was observed that Xolair treatment for 56 days reduced EAR 40% (placebo was unchanged). This finding confirmed the report of Boulet. Furthermore, in the first direct demonstration of the essential role of IgE in the LAR, Fahy observed a 63% improvement in the cellular inflammatory late phase following Xolair treatment.

The first direct study of the contribution IgE makes to the clinical state of moderate-severe asthma using the Xolair anti-IgE probe was reported by Milgrom et al. (14). Three hundred and seventeen subjects (age range 11–50 years) who required inhaled or oral corticosteroids (or both) were randomly assigned to receive either placebo or one of two regimens of Xolair intravenously for 20 weeks. For the first 12 weeks of the study, the subjects continued the regimen of corticosteroids they had received before enrollment. During the following 8 weeks, the doses of corticosteroids were tapered in an effort to discontinue this therapy. After 12 weeks of therapy, the mean symptom scores were improved by 30%

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($p = 0.005$). Twenty weeks of Xolair resulted in greater decreases and discontinuations of corticosteroids in the Xolair patients without a worsening of asthma control (14).

Together with colleagues across the United States and in Europe, we have designed, conducted, and analyzed large multicenter, double-blind, placebo-controlled trials confirming the initial observations of Boulet, Fahy, and Milgrom. The aim here, in this text, is to provide a review of three additional anti-IgE clinical trials: the first conducted exclusively in an adult U.S. asthmatic population, the second a wider study including international centers, and finally a study of anti-IgE (Xolair/omalizumab) in subjects with more severe asthma requiring high-dose corticosteroid treatment. We believe that the evidence en toto of the role of IgE in adult asthma mustered from cell culture, immunochemistry, population-based studies, and more specific clinical interventions with Xolair makes possible an incontrovertible conclusion.

II. U.S. and International Studies of Xolair Administered Subcutaneously to Moderate-Severe Asthmatics

U.S. subjects were enrolled using a 1:1 randomization in a 7-month double-blind core study comprised of 16 weeks of stable treatment followed by 12 weeks of attempted steroid dose reduction (Fig. 1). Patients received Xolair based on body weight and the total serum IgE level at the first visit (Fig. 2). The primary efficacy variables were the number of asthma exacerbation episodes experienced by a patient during the steroid-reduction period and during the double-blind stabilization period. There were a number of secondary efficacy variables. The secondary efficacy variables measured for the double-blind stabilization period were number of patients experiencing at least one exacerbation and the number of puffs of rescue medication taken. Secondary efficacy variables for the double-blind steroid-

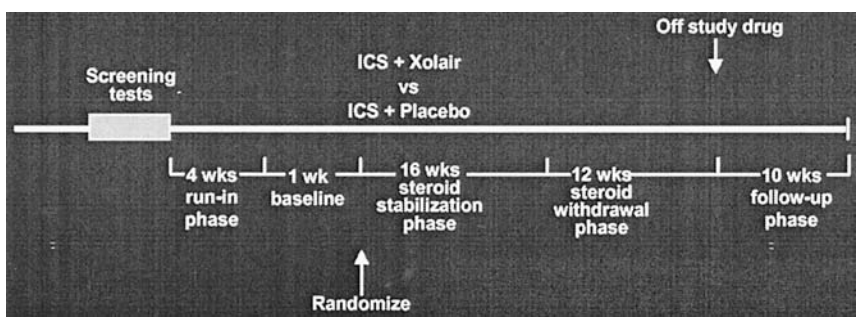

Figure 1 Xolair asthma study design.

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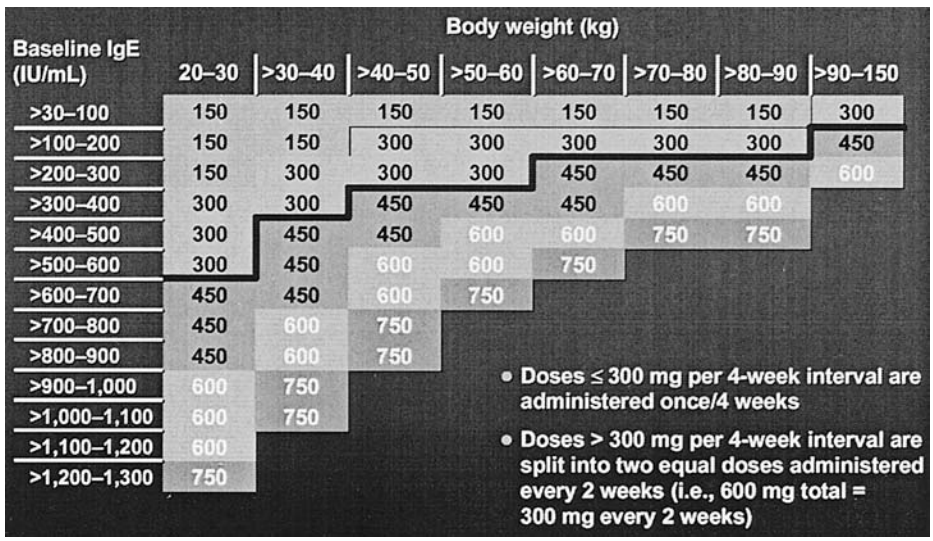

Figure 2 Xolair customized dosing for asthma.

reduction phase were percent reduction of the inhaled corticosteroid beclomethasone (BDP) and the number of patients having at least one asthma exacerbation. Exploratory variables measured during the double-blind stabilization period included morning PEFR, FEV_1, and total and individual asthma symptom scores (nocturnal, morning, and daytime).

Patients were enrolled with the diagnosis of asthma of $\geq$1 year duration, who, in addition to the standards of the American Thoracic Society (ATS) (15), demonstrated a $\geq$12% increase in FEV_1 following inhaled bronchodilator, skin prick test positivity (house dust mite [*D. farinae, D. pteronyssinus*], cockroach, dog, or cat) and remained symptomatic despite inhaled corticosteroids and frequent use of short-acting bronchodilators. Five hundred and twenty-five subjects were enrolled, 79% of whom had moderate disease severity and 21% of whom were judged to be severe (Table 1).

Both the investigator's assessed and the protocol-defined asthma exacerbation events (AEEs) occurred less frequently in Xolair-treated patients than placebo-treated ones. During 16 weeks of stable steroid treatment, the mean number of the protocol-defined AEEs (i.e., AEEs requiring systemic steroid burst) in Xolair-treated patients was almost one half of that in placebo-treated patients (0.28 vs. 0.54; $p = 0.006$). The number of patients experiencing AEEs (one or more) was approximately one third lower in the Xolair group than in the placebo group (14.6% vs. 23.3%, $p = 0.009$). Moreover, the evaluation of asthma symp-

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Table 1 U.S. Asthma Clinical Trial: Summary of Demographic and Baseline Characteristics by Treatment (all randomized patients)

	RhuMAb-E25 overall ($N = 268$)	Placebo overall ($N = 257$)	All patients ($N = 525$)
Sex, *n* (%)			
Male	104 (38.8)	111 (43.2)	215 (41)
Female	164 (61.2)	146 (56.8)	310 (59)
Race, *n* (%)			
Caucasian	238 (88.8)	229 (89.1)	467 (89.0)
Black	21 (7.8)	16 (6.2)	37 (7.0)
Other	9 (3.4)	12 (4.7)	21 (4.0)
Age group, *n* (%)			
12–17 years	20 (7.5)	21 (8.2)	41 (7.8)
18–64 years	241 (89.9)	229 (89.1)	470 (89.5)
$\geq$ 65 years	7 (2.6)	7 (2.7)	14 (2.7)
Mean age, year	39.3	39.0	39.2
(range)	(12–73)	(12–74)	(12–74)
Mean duration of asthma, years	20.56	22.65	21.58
(range)	(1–61)	(2–60)	(1–61)
Smoking status			
Never smoked	204 (76.1)	181 (70.4)	385 (73.3)
Ex-smoker	64 (23.9)	76 (29.6)	140 (26.7)
Mean BDP dose, μg/day	569.51	567.74	568.64
(range)	(420–1008)	(336–840)	(336–1008)
Mean serum total IgE, IU/mL	172.49	186.34	179.27
(range)	(20–860)	(21–702)	(20–860)
Mean FEV_1, % predicted	68.19	67.67	67.94
(range)	(30–112)	(32–111)	(30–112)
Mean qualifying FEV_1 reversibility, (%)	26.89	25.88	26.40
Severity of asthma, *n* (%)[a]			
Moderate	210 (78.4)	204 (79.4)	414 (78.9)
Severe	58 (21.6)	53 (20.6)	111 (21.1)

[a] Patients with FEV_1 percent of predicted at visit 3 less than 65% with average total symptom score during the 14 days prior to visit 3 greater than 4 are severe; otherwise, they are moderate.

toms, rescue medication use, and lung spirometric functions revealed a progressive improvement. The Xolair group had consistently lower mean asthma symptom scores, used lower number of puffs of rescue albuterol medication, and had slightly higher PEFR and FEV_1 than the placebo group; these treatment differences were generally statistically significant.

After 16 weeks of treatment, Xolair-treated patients were able to tolerate a significantly greater reduction in inhaled corticosteroid (ICS) dose than placebo

patients. The median percent reduction in the BDP dose was 75% in the anti-IgE group compared to 50% in the placebo group ($p < 0.001$). It was possible to withdraw BDP completely (100% dose reduction) in 39.5% of Xolair patients versus 19.1% of placebo patients. This reduction of BDP dose on Xolair was achieved without precipitating asthma exacerbations or an increase in rescue medication (both lessened on Xolair while decreasing coticosteroids). During the 12-week steroid-reduction phase, despite reduction in the BDP dose, Xolair was significantly superior to placebo in reducing the frequency of protocol-defined AEEs (mean 0.39 vs. 0.66 per patient; $p = 0.003$) and number of patients experiencing the AEEs (21% vs. 32%, $p = 0.004$). Significant reduction in potentially serious AEEs and corticosteroid requirement are important clinical benefits of this anti-IgE treatment.

Lung function assessments in the U.S. study revealed a small, statistically significant difference favoring Xolair. At the end of the stabilization phase, Xolair patients had a higher least-squares mean FEV_1 (2504 vs. 2381 mL) and morning PEFR (342 vs. 330L/min) than the placebo group. Considering the long duration (Table 1; mean duration 22 years) of the moderate to severe asthma and background maintenance therapy with moderately high doses of ICS while on study and frequent bronchodilator rescue, these increases in FEV_1 and PEFR, although small, are considered important evidence of the broad activity of Xolair anti-IgE.

The overall effectiveness of Xolair treatment was clearly reflected in measures of the asthma-related quality of life. Patient's self-assessment of asthma-related quality of life, using the validated asthma-related quality-of-life questionnaire (AQLQ) (16), showed a clinically significant change from baseline of 0.5 units improvement in both treatment groups for all domains at the end of both treatment stabilization and steroid-reduction periods. Importantly, a significantly greater improvement over placebo was seen in Xolair-treated patients for each of the individual domains (activities, emotions, symptoms, environmental exposure) and overall scores ($p = 0.02$).

The results of the safety analyses in this 7-month double-blind study showed that Xolair was well tolerated. There were no drug-related serious adverse events or other significant drug-related adverse events. The majority of adverse events were mild to moderate and considered not related to the study drug. Frequency of adverse events, whether or not drug related, was similar in the active and the placebo groups. The untoward reactions reported frequently in both treatment groups represent commonly occurring medical conditions observed in this adolescent and adult population; upper respiratory tract infection, viral infection, and headache were the most frequently reported in both treatment groups. Urticarial reactions occurred less frequently in the Xolair group than the placebo group (1.5% vs. 3.1%). These urticaria were typically mild to moderate in severity and were not considered to be drug related in any of the patients. The onset time of

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these urticarial reactions is not consistent with an immediate Type I hypersensitivity reaction. In all cases the urticaria resolved spontaneously or with antihistamine treatment and did not recur with continued Xolair treatment. There was no evidence of clinically significant abnormalities in the laboratory tests (chemistry, hematology, urinalysis) following Xolair treatment.

A second phase III, 7-month randomized, double-blind, parallel group, placebo-controlled multicenter trial conducted internationally confirmed the safety and efficacy of Xolair anti-IgE treatment in moderate-severe adult asthmatics. The 28-week double-blind treatment period was, once again, divided into a 16-week stabilization period during which Xolair or placebo were added to symptomatic patients requiring stable doses of BDP (500–1200 μg/day) followed by a 12-week steroid reduction phase (Fig. 1). Five hundred and forty-six patients were randomized (1 : 1 placebo : active) and were dosed according to serum IgE/body weight strata as in the U.S. trial (Fig. 2). Primary and secondary efficacy measures for this anti-IgE were unchanged. The study demographics and baseline clinical characteristics of the enrolled study subjects (Table 2) reveal a higher mean value for daily use of inhaled corticosteroids in this international study. To ensure a moderate to severe asthma population, all patients in this study were required to be symptomatic asthmatics and have an FEV_1 of no greater than 80% of predicted despite use of at least moderate doses of ICS (minimum beclomethasone 500 μg/day).

There were two primary endpoints: to assess the number of asthma exacerbations per patient during the stabilization and the steroid reduction periods of the study. As there is no widely accepted uniform guideline definition of asthma exacerbation, this study defined two types of AEEs. The investigator's clinical judgment of need for additional treatment, over and above the maintenance BDP dose and, as needed, β_2-agonist rescue medication, defined an exacerbation referred to as the ''investigator's assessed AEEs.'' To strengthen its clinical relevance, only those AEEs that required a corticosteroid burst (doubling of baseline maintenance BDP dose or oral/injected steroid) were included in the efficacy analyses (''protocol-defined AEE''). To ensure uniform evaluation of exacerbations across centers, patients were required to be evaluated by the study physician upon meeting any of the protocol-defined symptomatic criteria indicating a possible exacerbation. The physician was then directed to treat the patients according to published National Heart, Lung, and Blood Institute (NHLBI) guidance (17).

On average, Xolair resulted in 58% fewer asthma exacerbations per patient during the stabilization period for the intent-to-treat population ($p < 0.001$) than in the placebo group. Additionally, a significant reduction in exacerbation rates was duplicated in the second phase (52% fewer on average; $p < 0.001$) during the steroid-reduction period despite a greater reduction of inhaled corticosteroid use in the Xolair group. The observed reduction in these potentially serious exacerbations is considered a major clinical benefit of this new therapy. Indeed, there

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Table 2 International Asthma Clinical Trial: Summary of Demographic, Background, and Baseline Characteristics by Treatment (all randomized patients)

	rhuMAb-E25 (N = 274)	Placebo (N = 272)	All patients (N = 546)
Sex, *n* (%)			
Male	141 (51.5)	127 (46.7)	268 (49.1)
Female	133 (48.5)	145 (53.3)	278 (50.9)
Race, *n* (%)			
Caucasian	256 (93.4)	242 (89.0)	498 (91.2)
Black	11 (4.0)	11 (4.0)	22 (4.0)
Oriental	2 (0.7)	6 (2.2)	8 (1.5)
Other	5 (1.8)	13 (4.8)	18 (3.3)
Age, *n* (%)			
12–17 years	18 (6.6)	17 (6.3)	35 (6.4)
18–64 years	237 (86.5)	246 (90.4)	483 (88.5)
≥65 years	19 (6.9)	9 (3.3)	28 (5.1)
Age (yr), mean (range)	40.0 (12–76)	39.0 (12–72)	39.5 (12–76)
Duration of asthma (yr)[a] mean (range)	20.3 (2–68)	19.1 (1–63)	19.7 (1–68)
Smoking status, *n* (%)			
Nonsmoker	213 (77.7)	207 (76.1)	420 (76.9)
Ex-smoker	61 (22.3)	65 (23.9)	126 (23.1)
BDP dose (μg/day)			
Mean	769.0	772.1	770.5
Range	500–1600	200–2000	200–2000
Serum total IgE (IU/mL)			
Mean	223.1	205.6	214.4
Range	21–785	22–814	21–814
% predicted FEV_1			
Mean	69.8	69.9	69.8
Range	30–112	22–109	22–112
Qualifying FEV_1 reversibility (%)[b]			
Mean	26.4	25.8	26.1
Range	10–86	11–103	10–103
Severity of asthma, *n* (%)[c]			
Moderate	214 (78.1)	213 (78.3)	427 (78.2)
Severe	60 (21.9)	59 (21.7)	119 (21.8)
Past year hospital or doctor visits for asthma:			
Overnight hospital admission, *n* (%) patients	11 (4.1)	20 (7.5)	31 (5.8)
Emergency room visits, *n*, mean (range)	0.23 (0–12)	0.17 (0–6)	0.20 (0–12)
Doctor's office visits, *n*, mean (range)	1.18 (0–15)	1.21 (0–24)	1.20 (0–24)
Missed work or school days, *n*, mean (range)	4.34 (0–190)	2.82 (0–60)	3.60 (0–190)
History of:[a]			
Atopic dermatitis, *n* (%) patients	24 (8.8)	28 (10.3)	52 (9.5)
Seasonal allergic rhinitis, *n* (%)	183 (66.8)	177 (65.1)	360 (65.9)
Perennial allergic rhinitis, *n* (%)	213 (77.7)	209 (76.8)	422 (77.3)
Aeroallergens sensitivity, *n* (%)	232 (84.7)	221 (81.3)	453 (83.1)
Foods sensitivity, *n* (%)	62 (22.6)	58 (21.3)	120 (22.0)
Animals sensitivity, *n* (%)	225 (82.1)	227 (83.5)	452 (82.9)
Dust mite sensitivity, *n* (%)	247 (90.1)	246 (90.4)	493 (90.5)

[a] Missing asthma and allergen history CRF for one patient: 21/2236 on placebo q2weeks.

[b] Missing qualifying FEV_1 reversibility for one patient: 33/2934 on placebo q2weeks.

[c] Moderate: % predicted FEV_1 at visit 3 (baseline) >65%; severe: % predicted $FEV_1 \leq 65\%$ and a mean total symptom score of >4 for the 14 day-period preceding visit 3.

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were six patients (2.2%) in the placebo group versus none in the Xolair group who experienced asthma exacerbations judged as serious adverse events.

Results of the secondary efficacy variables (number of patients experiencing at least one asthma exacerbation, percent reduction in the dose of BDP, proportion of patients with reduction in BDP, number of puffs of rescue medication taken daily, and global evaluations of treatment effectiveness) were consistent with the Xolair superiority seen in the primary endpoint. Furthermore, consistency was also seen in the exploratory variables including PEFR, spirometry, and mean asthma symptom scores, with statistically significant differences shown in favor of anti-IgE for most variables.

Of particular note was the clinically meaningful improvement in:

- Nocturnal asthma symptoms (median score falling from 1.14 to 0.36 after 16 weeks of Xolair treatment, where a score of 1 represents a daily awakening due to asthma symptoms, compared to a change from 1.29 to 0.83 on placebo)
- The reduction of inhaled corticosteroids (the median BDP dose decrease was 83% on Xolair versus 50% in placebo plus standard therapy patients)
- Rescue β_2-agonist use (a decrease of 2 puffs/day after 16 weeks of treatment compared to 0.6 puffs reduction achieved on placebo plus standard therapy)

Importantly, the benefit of reduced asthma exacerbations seen in the Xolair group during the stabilization period continued during the steroid-reduction phase despite a significantly greater reduction of inhaled corticosteroid and less frequent use of rescue medication. The evidence of superior symptom control and respiratory function, which also continued throughout the steroid-reduction period, further enhances the confidence in these anti-IgE efficacy results.

The observed safety profile of Xolair was excellent, and the subcutaneous treatment was well tolerated in this large population of moderate to severe adult and adolescent asthmatics during 28 weeks of exposure. There were no drug-related serious adverse events in the core study period (16 weeks). The number of patients who had at least one adverse event, whether or not study drug related, was similar in both treatment arms (Xolair 80.7% vs. 78.3% placebo), viral infection, headache, and sinusitis being the most frequently reported events in both groups. There was no significant difference between the number of Xolair- and placebo-treated patients experiencing at least one adverse event suspected as drug related (6.2% vs. 3.7%, respectively; $p = 0.183$). Urticaria occurred in one (0.4%) Xolair patient versus four (1.5%) placebo-treated patients. Local injection site symptoms were associated with 11.8% of Xolair injections compared to 7.7% of placebo administrations. No patients discontinued treatment due to adverse events in the Xolair group compared to five (1.8%) on placebo. There were no cases of anaphylaxis or serum sickness during the treatment period. One case of protein-

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uria occurred in a patient receiving anti-IgE. From follow-up information, this was not considered drug related, but instead was attributed to mild hypertension. A renal biopsy gave no indication of immune complex disease.

This study demonstrates that treatment with anti-IgE reduces the risk of potentially serious asthma exacerbations and improves asthma control in patients with moderate to severe allergic asthma. It adds further weight to the accumulated evidence for the key pathogenetic role IgE plays in patients with asthma.

III. Study of Xolair Anti-IgE in Severe Asthma

This study was conducted in 15 extra-U.S. cities, which made possible accrual of adolescents and adults with severe asthma requiring daily treatment with high-dose inhaled corticosteroids ($\geq$1000 mg/d fluticasone) often concomitantly treated with oral corticosteroids. Patients 12–75 years old were enrolled if treated with oral corticosteroids for $>$5 years only if reversibility to salbutamol was demonstrable; 341 patients were randomized (Xolair 176/placebo 165) and dosed subcutaneously with the anti-IgE as in previous late stage clinical trials (Fig. 2). The primary efficacy variable was the percentage reduction in ICS dose at the end of a 32-week treatment phase compared to baseline. Key secondary efficacy measures included change from baseline in mean daily dose of oral steroid and the number of patients experiencing at least one AEE. The background characteristics by steroid use (inhaled/oral) and treatment randomization (Xolair/placebo) are presented in Table 3. There were no marked differences in demographics between the inhaled and oral groups or between study treatments. A paucity of younger patients in the oral corticosteroid subpopulation may reflect prudent avoidance of systemic glucocorticoids in younger asthmatics (18,19). Note that 58–67% of subjects were female, which is expected in a study of severe asthmatics requiring high doses of corticosteroids (20,21). It may be that the preponderance of asthmatics treated with ICS and randomized to receive Xolair (Table 3, column 1) had more severe disease as indicated by lower baseline FEV_1, a higher incidence of hospitalization, and more prevalent history of atopic dermatitis comorbidity.

A. Reduction in Corticosteroids

For all randomized patients in the inhaled steroid subpopulation, percent reductions in fluticasone dose at the end of double-blind treatment (week 32) are summarized in Table 4. For the inhaled randomized patients subpopulation, there was a highly significant reduction in inhaled steroid dose at the end of the treatment phase in E25 patients compared to placebo ($p = 0.003$). Additionally, the median percent reduction was greater for E25 patients compared to placebo patients in both the Q2 week and Q4 week dosing subgroups. The distribution of

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Table 3 Severe Asthma: Demographic and Background Characteristics by Steroid and Treatment Group (all randomized patients)

	Inhaled		Oral		Overall		All patients ($N = 341$)
	E25 ($N = 126$)	Placebo ($N = 120$)	E25 ($N = 50$)	Placebo ($N = 45$)	E25 ($N = 176$)	Placebo ($N = 165$)	
Sex, *n* (%)							
Male	45 (35.7%)	51 (42.5%)	18 (36.0%)	15 (33.3%)	63 (35.8%)	66 (40.0%)	129 (37.8%)
Female	81 (64.3%)	69 (57.5%)	32 (64.0%)	30 (66.7%)	113 (64.2%)	99 (60.0%)	212 (62.2%)
Race, *n* (%)							
Caucasian	106 (84.1%)	101 (84.2%)	40 (80.0%)	35 (77.8%)	146 (83.0%)	136 (82.4%)	282 (82.7%)
Black	0	2 (1.7%)	0	1 (2.2%)	0	3 (1.8%)	3 (0.9%)
Oriental	2 (1.6%)	1 (0.8%)	0	0	2 (1.1%)	1 (0.6%)	3 (0.9%)
Other	18 (14.3%)	16 (13.3%)	10 (20.0%)	9 (20.0%)	28 (15.9%)	25 (15.2%)	53 (15.5%)
Age, *n* (%)							
12–17 years	12 (9.5%)	9 (7.5%)	0	0	12 (6.8%)	9 (5.5%)	21 (6.2%)
18–64 years	104 (82.5%)	106 (88.3%)	46 (92.0%)	40 (88.9%)	150 (85.2%)	146 (88.5%)	296 (86.8%)
≥65 years	10 (7.9%)	5 (4.2%)	4 (8.0%)	5 (11.1%)	14 (8.0%)	10 (6.1%)	24 (7.0%)
Age (yr) mean (range)	41.1 (12–75)	40.5 (12–71)	46.6 (18–71)	47.8 (19–74)	42.7 (12–75)	42.5 (12–74)	42.6 (12–75)
Duration of asthma (yr) mean (range)	22.6 (2–70)	22.3 (1–64)	21.6 (2–62)	22.1 (1–60)	22.3 (2–70)	22.3 (1–64)	22.3 (1–70)
Never smoked, *n* (%)	99 (78.6%)	91 (75.8%)	37 (74.0%)	32 (71.1%)	136 (77.3%)	123 (74.5%)	259 (76.0%)
Ex-smoker, *n* (%)	27 (21.4%)	29 (24.2%)	13 (26.0%)	13 (28.9%)	40 (22.7%)	42 (25.5%)	82 (24.0%)

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Table 4 Percent Reduction in Inhaled Steroid (fluticasone) Dose at End of Treatment Phase (visit 15/week 32) Compared to Baseline (visit 3/week 0) (all randomized patients—inhaled)

	% Reduction in inhaled steroid dose						
	Q2 week		Q4 week		Inhaled		
	E25	Placebo	E25	Placebo	E25	Placebo	*p*-value
N	63	64	63	56	126	120	
Median	50.0	46.4	60.0	50.0	60.0	50.0	0.003*
Range	−33.3–100	−60–100	−75–100	−100–100	−75–100	−100–100	

* Significant at the 0.05 level. Comparison of E25 and placebo for inhaled steroid subpopulation. Generalized CMH (van Elteren) test using standardized midranks and controlling for dosing schedule.

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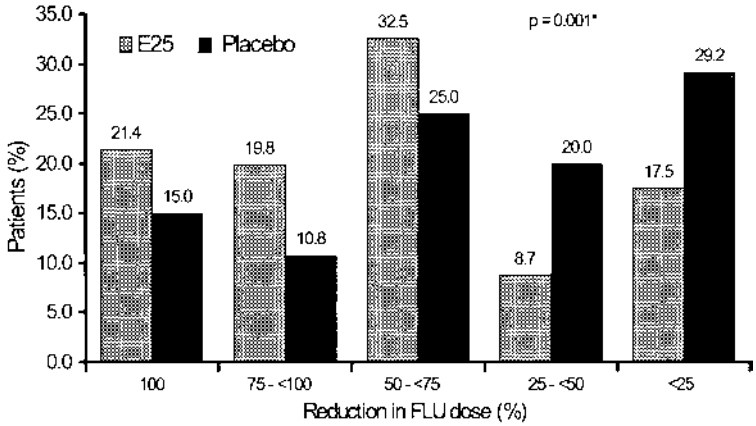

Figure 3 Percent reduction in steroid dose at the end of the treatment phase (visit 15/week 32) compared to baseline (visit 3/week 0) (all randomized patients—inhaled). FLU, fluticasone.

reduction (Fig. 3) revealed that 41.2% of Xolair-treated patients achieved a ≥75% reduction in CS (placebo 25.8%). The absolute reduction in fluticasone dose was also significantly greater for Xolair patients compared to placebo patients. For the oral CS randomized patient subpopulation, there were no between-treatment differences in percent reduction prednisolone dose.

B. Reduction in Exacerbations

A protocol-defined AEE was defined as a worsening of asthma (decreased a.m. PEFR, worsening symptom scores unresponsive to increased rescue med use) requiring treatment with new or increased doses of systemic oral corticosteroids. There was an increase in the number of placebo patients experiencing one or more AEE in the corticosteroid reduction phase compared to the stabilization phase. This increase was more marked in placebo patients (0.25 episodes/patient to 0.45) compared to Xolair-treated patients (0.23–0.27 AEE/patient, respectively) (Table 5).

C. Other Efficacy Measures of Anti-IgE Treatment in Severe Asthmatics

In the inhaled steroid subpopulation, secondary efficacy results were also in favor of Xolair, showing statistical significance versus placebo in lowering absolute steroid dose at the end of the treatment phase and improvements in rescue medication use, asthma symptoms, and lung function. Statistical significance was demonstrated in these parameters at a number of time points. Most of the secondary

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Table 5 Number of Asthma Exacerbation Episodes per Patient During the Double-Blind Stabilization and Double-Blind Steroid Reduction Phase (all randomized patients)

	E25	Placebo
Stabilization phase		
Number of AEE		
0 (n)	149	141
1 (n)	17	16
2 (n)	8	3
3 (n)	1	3
4 (n)	1	2
N	176	165
Median [range] AEE	0 [0–4]	0 [0–6]
Mean number AEE	0.23	0.25
Reduction phase		
Number of AEE		
0 (n)	130	117
1 (n)	26	29
2 (n)	5	8
3 (n)	3	2
4 (n)	0	3
N	164	159
Median [range] AEE	0 [0–3]	0 [0–9]
Mean AEE	0.27	0.45

objectives of the study were adequately addressed in the prospective analysis, but in some efficacy parameters unplanned analyses were employed for further investigation of the underlying data. Nighttime awakenings, use of rescue medication, and asthma symptom scores all suggest that baseline disease was more severe in patients randomized to Xolair. CS taper in the placebo group was accompanied by a worsening of symptoms and an increase in rescue medication during the steroid reduction phase (questioning the true benefit of steroid reduction in this group); there was no such penalty for steroid reduction in the Xolair group. With Xolair there was an improvement in asthma symptoms and a reduction in rescue medication use while achieving meaningful CS reduction.

Clinically meaningful improvements in quality-of-life (QOL) domains were seen in more Xolair-treated patients compared to placebo patients at the end of the stabilization and reduction phases. The improvement in activities, emotions, symptoms, and overall domains at the end of the reduction phase was seen

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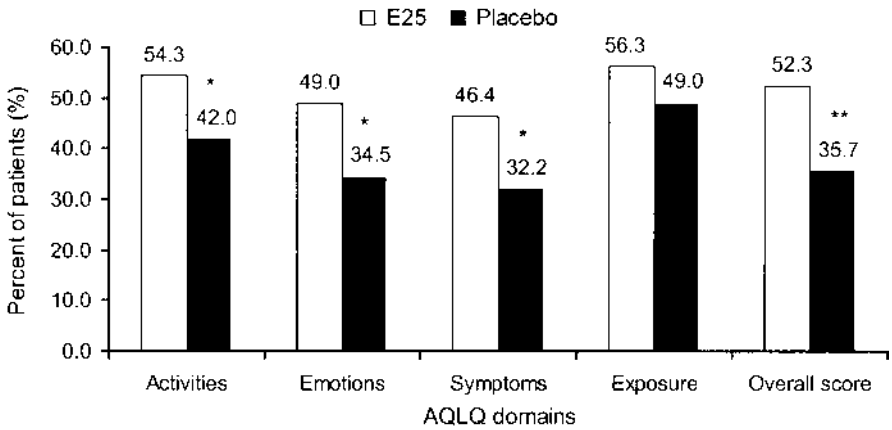

Figure 4 Clinically detectable (≥0.5) improvement in QOL scores at the end of the steroid-reduction phase (all randomized patients). Significance: *$p < 0.05$, **$p < 0.01$.

in significantly more Xolair patients (Fig. 4). In the oral subpopulation during the stabilization phase, asthma-related QOL was generally better in Xolair-treated patients, especially for the symptom domain. In the reduction phase oral-treated group, change in symptom score improved overall in the anti-IgE group and worsened in the placebo group. Emotional and overall domain scores improved more markedly in Xolair patients than in placebo patients.

D. Safety

The observed safety profile of Xolair continued to be excellent, and treatment was well tolerated in this population of severe asthmatics during 32 weeks of exposure. There were no drug-related serious adverse reactions in the double-blind period. The number of patients who had at least one adverse event, whether or not study drug related, was similar in both treatment arms, viral infection, headache, and rhinitis being the most frequently reported events in both groups. There was no meaningful difference between the number of Xolair- and placebo-treated patients experiencing at least one adverse event suspected as drug related. Clinically notable abnormalities in laboratory parameters were reported in few patients, were distributed equally across treatment groups, and did not give rise to any clinical concern.

The only area in the safety data where Xolair appeared to be inferior was in local symptoms associated with the study medication injections. Local symptoms were associated with 17.4% of Xolair injections compared to 10.2% of placebo administrations. Most of the local symptoms were mild and transient.

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Specific reactions like rash, hives, urticaria, pruritus, or allergic reactions were reported infrequently and, if anything, were more prevalent in the placebo group.

IV. Conclusions

The immunoglobulin isotype identified by Ishizaka and Johansson in 1967 has now progressed far in its journey of scientific discovery from the bench in polyacrylamide gels to the bedside, as demonstrated in successful multicenter trials of Xolair anti-IgE. The progression has been incremental and well reasoned, starting with immunochemistry, to short-term cell culture studies of effector cells and stimulated mediator release bolstered by a very different science: population-based studies associating serum IgE with asthma symptoms and airways reactivity. The dual conceptual breakthroughs of identification of the Fcε3 domain binding high-affinity receptor as a target for a therapeutic MAb (22) followed by humanization of the MAb (10) have made possible direct study of the specific role of IgE in the asthmatic airway. Specific binding of IgE by Xolair has been shown to safely improve early asthmatic response, dramatically reverse late asthmatic response, and offer important clinical improvement to adolescent and adults with moderate to the severest stages of asthma.

As with all scientific inquiry, the systematic process of answering a question often raises more intriguing questions. What is the contribution of serum IgE to the childhood risk of developing asthma (23,24)? Is IgE essential to the atopic march (25,26) from food allergies to atopic dermatitis eventuating in asthma? And now we can pose even grander questions: Would early Xolair intervention in at-risk children abrogate wheezy-asthma disease? Or in those asthmatics with established disease, is it possible that chronic Xolair anti-IgE treatment will modify the course of their lung disease? These trials have not yet been designed, but given their importance it is inevitable that in the not too distant future they will be undertaken. For the moment, it is exhilarating just phrasing these questions, knowing that a safe reagent exists with which to probe the unknown.

References

1. Ishizaka K, Ishizaka T. Identification of γE-antibodies as a carrier of reaginic activity. J Immunol 1967; 99:1187.
2. Johansson SGO, Bennich H. Studies on a new class of human immunoglobulins. I. Immunological properties. In. Killander J, ed. Gammaglobulins. Structure and Control of Biosysnthesis. Stockholm: Almqvist & Wiksell, 1967:193–197.
3. Brechter C, Rorsman H. Basophil leukocytes in hyposensitisation. Int Arch Allergy Appl Immunol 1965; 28:35–40.
4. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TA. Epidemiology of

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acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989; 325:1067–1071.
5. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989; 320: 271–277.
6. Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holday MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. N Engl J Med 1991; 325:1067–1071.
7. Sunyer J, Anto JM, Sabria J, Roca J, Morell F, Rodriguez-Roisin R, Rodrigo MJ. Relationship between serum IgE and airway responsiveness in adults with asthma. J Allergy Clin Immunol 1995; 95:699–706.
8. Gergen PJ, Turkeltaub PC, Kovar MG. The prevalence of allergic skin test reactivity to eight common aeroallergens in the U.S. population: results from the second National Health and Nutrition Examination Survey. J Allergy Clin Immunol 1987; 80: 669–679.
9. Ma X, Fick RB, Kaplowitz HJ. Asthma and Allergic Asthma in the United States: Prevalence and severity data from the Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994. Am J Resp Crit Care Med 2000; 161(3): 199.
10. Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, Jardieu PM. Humanization of an anti-body directed against IgE. J Immunol 1993; 151:2623–2632.
11. Saban R, Haak-Frendscho M, Zine M, Ridgway J, Gorman C, Presta LG, Djorling D, Saban M, Jardieu P. Human FcERI-IgE and humanized anti-IgE monoclonal antibody MaE11 block passive sensitization of human and rhesus monkey lung. J Allergy Clin Immunol 1994; 94:836–843.
12. Boulet LP, Chapmen KR, Cote J, Kalra S, Bhagat R, Swystun VA, Laviolette M, Cleland LD, Deschesnes F, Su JQ, DeVault A, Fick RB, Cockcroft DW. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. Am J Respir Crit Care Med 1997; 155:1835–1840.
13. Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, Fick RB, Boushey HA. The effect of an anti-IgE monoclonal antibody on the early and late phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med 1997; 155:1828–1834.
14. Milgrom H, Fick RB, Jr, Su JQ, Reimann JD, Bush RK, Watrous ML, Metzger WJ, for the rhuMAb-E25 Study Group. Treatment of allergic asthma with monoclonal anti-IgE antibody. N Engl J Med 1999; 341(26):1966–73.
15. American Thoracic Society Committee on Diagnostic Standards for Non-Tuberculous Respiratory Diseases: Definitions and clarifications of chronic bronchitis, asthma, and pulmonary emphysema. Am Rev Respir Dis 1962; 85:762–768.
16. Juniper EF, Guyatt GH, Willan A, Griffith LE. Determining a minimal important change in a disease-specific quality of life instrument. J Clin Epidemiol 1994; 47: 81–87.
17. National Institutes of Health and National Heart, Lung, and Blood Institute. Guidelines for the Diagnosis and Management of Asthma. Expert Panel Report 2. Clinical Practice Guidelines. Bethesda, MD: NIH publication 97-4051, 1997.

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18. Agertoft L, Pedersen S. Effects of long-term treatment with an inhaled corticosteroid on growth and pulmonary function in asthmatic children. Respir Med 1994; 88:373–381.
19. Lipworth BJ, Jackson CM. Safety of inhaled and intranasal corticosteroids: lessons for the new millennium. Drug Saf 2000; 23:11–33.
20. Chung KF, Godard P, Adelroth E, Ayres J, Barnes N, Barnes P, Bel E, Burney P, Chanez P, Connett G, Corrigan C, de Blic J, Fabbri L, Holgate ST, Ind P, Joos G, Kerstjens H, Leuenberger P, Lofdahl CG, McKenzie S, Magnussen H, Postma D, Saetta M, Salmeron S, Sterk P. Difficult/therapy-resistant asthma: the need for an integrated approach to define clinical phenotypes, evaluate risk factors, understand pathophysiology and find novel therapies. ERS Task Force on Difficult Therapy-Resistant Asthma. European Respiratory Society, Eur Respir J 1999; 13:1198–1208.
21. Leung DY, Spahn JD, Szefler SJ. Immunologic basis and management of steroid-resistant asthma. Allergy Asthma Proc 1999; 20:9–14.
22. Presta LG, Shields R, O'Connell L, Lahr S, Porter J, Gorman C, Jardieu P. The binding site on human immunoglobulin-E for its high affinity receptor. J Biol Chem 1994; 269(42):26368–26373.
23. Martinez FD, Wight AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. N Engl J Med 1995; 332:133–138.
24. Reijonen TM, Korppi M. One year followup of young children hospitalized for wheezing: the influence of early anti-inflammatory therapy and risk factors for subsequent wheezing and asthma. Pediatr Pulmonol 1998; 26:113–119.
25. Price GW, Duff AL, Farris AH, Platts-Mills TAE, Heymann PW. IgE antibody responses to common food allergens in wheezing infants and children. J Allergy Clin Immunol 1993; 91:173.
26. Clough JB, Williams JD, Holgate ST. Effect of atopy on the natural history of symptoms, peak expiratory flow, and bronchial responsiveness in 7- and 8-year-old children with cough and wheeze. Am Rev Respir Dis 1991; 143:755–760.

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12

The Role of IgE in Pediatric Asthma

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I. Introduction

Asthma is a complex multifactorial disease in which allergic sensitization plays an essential role. Sensitivity to indoor allergens is associated with prevalence, severity, and exacerbations of asthma, while exposure to outdoor allergens initiates exacerbations (1,2). A trait for an elevated level of serum IgE is coinherited with bronchial hyperresponsiveness. Thus, there is a second link between IgE and airway inflammation that appears to be independent of specific allergic susceptibility (3).

Serum IgE levels correlate with the severity of clinical manifestations of asthma (4,5). During childhood total serum IgE appears to track with age. Infants less than one year old with high serum IgE continue to have elevated levels at 6 and 11 (6) (Fig. 1). These children are predisposed to persistent wheezing and early sensitization to aeroallergens. Others, with wheezing only during the first year of life and not later, have serum IgE concentrations similar to those of nonwheezing children.

Wheezy infants who will have asthma are difficult to distinguish from others whose symptoms will be transient. A vast majority of all cases of asthma have their onset in the first years of life (7), but no more than a minority of

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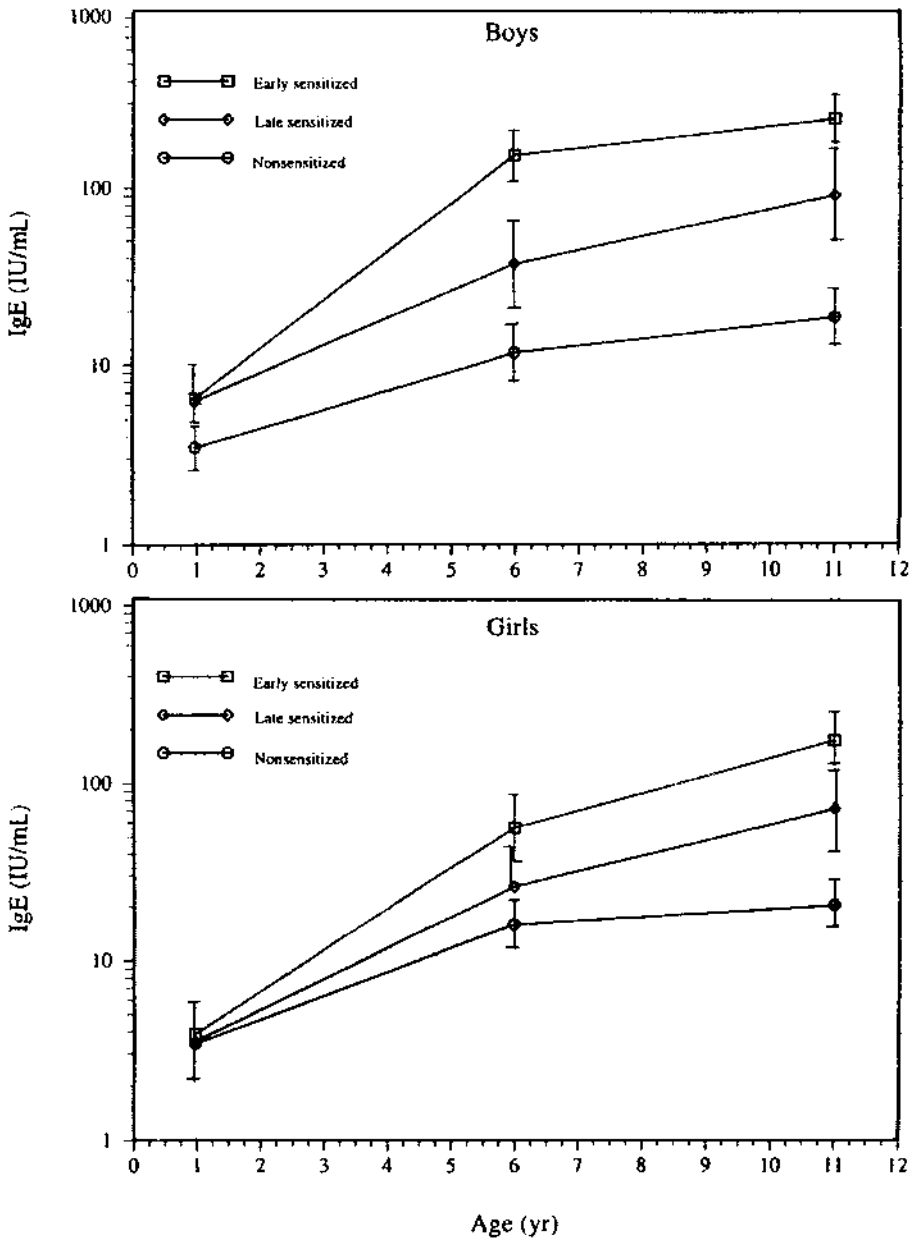

Figure 1 Mean IgE and 95% confidence intervals for model 1 with repeated-measures analyses (see text for details) for boys and girls. Children were considered early sensitized when they had at least one positive skin prick test response at age 6 years and late sensitized when they had at least one negative skin prick test response followed by a positive skin prick test response at age 11 years. Nonsensitized children had negative skin prick test responses at both ages (reference population). (From Ref. 6.)

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infants who wheeze have or will proceed to develop this disease (8,9). Despite early similarities in lung function, children who had a wheezing lower respiratory tract illness in the first 3 years and whose wheezing episodes persisted up to the age of 6 had inferior lung function at that age in comparison to others whose wheezing started after age 3 (10). The children with early onset of asthma symptoms had greater deterioration in lung function and more persistence of symptoms into adulthood (7,11).

One feature of asthma that appears to be particularly important is the thickening of the basement membrane of the airway epithelium and the deposition of collagen and other matrix products in the airway wall. It has been suggested that these changes constitute the basis for remodeling of the airway, a process that may predispose to airway hyperresponsiveness and to the development of chronic, persistent asthma symptoms. Thus, therapeutic strategies for wheezy infants must address the possibility that for those who will go on to develop asthma, a prolonged delay in anti-inflammatory treatment leads to permanent loss in pulmonary function.

II. Etiology and Epidemiology of Pediatric Asthma

The susceptibility to allergic disease is determined by a combination of genetics and environment acting through a complex network of cytokines (Fig. 2). It is sustained by an imbalance between two populations of T-helper lymphocytes referred to as Th1 and Th2 cells, with the equilibrium shifted toward Th2. Interleukin-4 (IL-4) is pivotal in Th2 cell development (12) and, together with IL-13, leads to increased IgE production. Both cytokines can upregulate adhesion molecule expression and mucus production in the airways. IL-5 is thought to be

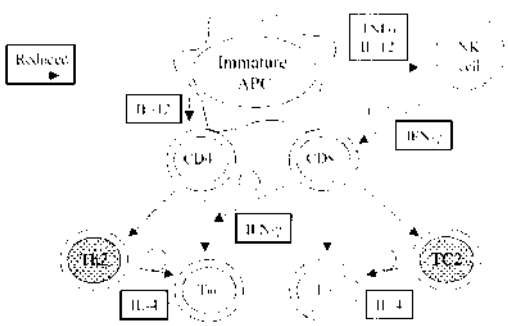

Figure 2 Theoretical development of TC2/Th2-like responses. In an immature APC system antigen presentation without IL-12 promotes the development of Th2-like and TC2-like effector cells. (From Ref. 64.)

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essential for eosinophilopoiesis and eosinophil survival. Atopic disorders caused by Th2-like immune responses directed against otherwise harmless antigens are on the rise in affluent societies. In the developing world common infections limit the prevalence of allergic disorders by inducing a Th1 immunological environment rich in interferon gamma (IFN-γ). Exposure to microorganisms in infancy suppresses allergen-specific Th2 cytokines and the emergence of atopy. However, when infections occur after the Th2 response is already established, IL-4 converts the normally dominant Th1 response into a mixed Th1/Th2 process, resulting in an exacerbation of the atopic disorder. Thus the increase in prevalence and severity of atopic disorders in prosperous societies may be caused in part by the decrease in infectious diseases in early childhood (13).

Th2 cells infiltrate the airway wall as a result of attraction to inflammatory sites by adhesion molecules and chemokines. Production of Th2 cytokines is not restricted to T cells. Both basophils and mast cells produce IL-4, suggesting that these cells may bear upon the synthesis of IgE (2). IgE production by B cells requires not only the presence of IL-4 or IL-13, but also a physical interaction between T and B cells involving surface and adhesion molecules. IgE, bound to low-affinity receptors (FcεRII [CD23]) on activated B cells and antigen-presenting cells such as monocytes and Langerhans cells, appears to enhance their antigen-capturing activity and the production of still more IgE (14,15).

The prevalence of atopy and asthma has been on the rise in affluent countries (16) (Table 1). Interestingly, both conditions remain uncommon in the less developed areas, especially in rural regions (17). Comparisons drawn between children raised in cities and those growing up in traditional surroundings in the third world have shown a marked increase in the incidence of asthma among urban dwellers. The risk of developing allergic disease appears to be related to socioeconomic factors and family structure (18). In societies as dissimilar as the United Kingdom and Ghana, the less affluent groups are at lower risk for atopic disease (19,20). Inverse relationships have been reported between atopy and the number of siblings (21) and between atopy and early entry into communal day-care (22).

Extensive information about wheezy children has been accumulated by the Tucson Children's Respiratory Study. This long-term, longitudinal, prospective inquiry into the risk factors for acute lower respiratory tract illnesses in early childhood and for chronic obstructive airways disease in later life was based on the surveillance of 1246 newborns enrolled between May 1980 and January 1984, representing 78% of eligible infants (23). When the children turned 6 they were classified into groups according to the presence or absence of wheezing before or after they reached 3 years of age (10). Four hundred and twenty-five children (51.5%) never wheezed, 19.9% had transient early wheezing, 15% had wheezing of late onset, and 13.7% had persistent wheezing. Thus, the great majority of the cohort was not wheezing by 6 years of age. Those who continued to have recur-

Table 1 Estimated Average Rates[a] of Office Visits for Asthma as the First-Listed Diagnosis, by Race, Sex, and Age Group—United States, National Ambulatory Medical Care Survey, 1975–1995[b]

	1975	1980–1981	1985	1989	1990–1992	1993–1995
White	22.2	26.2	29.3	26.2	34.6	39.6
Black	19.7[c]	22.4[c]	26.8	29.9	39.5	43.8
Other	[d]	[d]	[d]	[d]	17.3	34.1
Male	21.1	25.8	27.3	21.0	31.2	33.9
Female	21.5	23.7	28.3	32.7	36.7	43.6
Age group (yr)						
0–4	25.3[c]	30.4[c]	30.7	32.7[c]	48.2	50.3
5–14	22.5	45.6	42.6	27.0	49.3	51.5
15–34	14.1	13.9	14.6	19.0	23.9	22.8
35–64	25.4	20.7	28.8	31.7	29.5	41.7
≥65	25.3	25.8	33.0	30.9	37.0	44.0
Total	211.4	25.0	27.9	27.0	34.1	39.0

[a] Per 1000 population.

[b] All relative standard errors are <30% (i.e., relative confidence interval < 59%) unless otherwise indicated.

[c] Relative standard error of the estimate is 30–50%; the estimate is unreliable.

[d] Relative standard error of the estimate exceeds 50%.

rent symptoms had had more episodes early in their lives than those whose symptoms ceased by the age of 3; moreover, they experienced episodes of wheezing even in the absence of viral infection. The children with persistent wheezing were more likely to have had a history of atopy and to have been the offspring of mothers with asthma. Maternal smoking was associated with both persistent and transient wheezing. More recently, specific allergen reactivity and elevated IgE have been found to be associated with persistent wheezing (24).

Alterations in acute immune response to viral infection may be detected at the time of the first wheezing episode in subjects who will go on to have persistent wheezing symptoms (10,25,26). Viral respiratory infections early in life appear unlikely to give rise to persistent asthma, although they may trigger acute wheezing illnesses (24,26). However, the presence of eosinophil activation in the peripheral blood at the time of bronchiolitis in infants is linked to an increased risk of wheezing at age 7 (26). Eosinophilia may identify a predisposition toward Th2 dominance that provokes airway obstruction at the time of viral infection in infancy and during allergen exposure in later life.

Those children who are more likely to have asthma appear to show evidence of an altered immunological process, i.e., they become sensitized to allergens and other antigens early in life. The association among environmental allergens,

IgE-mediated hypersensitivity, and asthma is supported by the following evidence:

- The initial onset of wheezing is related to the density of house dust mite allergen, *der p* 1, exposure in infancy (27).
- The onset of asthma has been related to the sensitization to only a handful of allergens: dust mite, animal dander, cockroach, and the mold *Alternaria* (28).
- Sensitization, with production of IgE antibodies, is a strong risk factor for acute asthma, especially when these sensitized individuals are exposed to high concentrations of allergen in their homes (4,29–31).
- Asthma severity and airway hyperresponsiveness correlate with the degree of sensitivity to indoor allergens (27,32,33).
- Sixty to 80% of adults and children with asthma have one or more positive immediate wheal-and-flare skin test responses to environmental allergens (32,34–36).
- Inhaled extracts of aeroallergens can induce asthma in sensitized subjects (37,38).
- Asthma symptoms and measures of lung function improve in sensitized patients when they avoid aeroallergens (39–42).

III. Role of IgE in the Pathogenesis of Asthma

There appears to be a strong causal relationship between allergy and the origins of asthma (12). In children this disease is strongly linked to the development of specific IgE. Nearly 90% of affected children have positive skin tests, with the indoor allergens house dust mite, *Alternaria*, cockroach, and cat most closely linked with the disease (1,28). There is a strong correlation between the number of positive skin tests in children and the severity of asthma (43). Greater exposure to house dust mite protein during infancy has been shown to lead to an earlier onset of wheezing (44). Finally, total IgE and atopy manifested by positive skin tests are the main determinants of bronchial hyperesponsiveness in young adults (45).

The IgE-mediated reactions of a sensitized asthmatic following the exposure to an allergen have been designated as early (EPR) and late phase responses (LPR) (46). EPR is manifested by a fall in forced expiratory volume in 1 second (FEV_1) within one hour of allergen exposure (46,47). EPR is initiated when IgE molecules bound to high-affinity receptors (FcεRI) on the surface of mast cells and basophils are cross-linked by allergen. This results in cellular degranulation and the release of preformed inflammatory mediators. Mast cell degranulation in vivo is often followed by LPR. IgE antibodies are capable of passive transfer of both acute and LPR sensitivity to allergen challenge (48). In asthmatics, LPR

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appears as a second episode of airflow obstruction 4–8 hours after antigen exposure. The intensity of the airway obstruction that develops during the LPR is more prolonged, and usually more severe, than that observed during the EPR.

Chronic airway symptoms may result from persistent LPR to repeated allergen exposure (48). LPR shares determinant factors with EPR. IgE-mediated mast cell activation gives rise to both reactions (48). Patients who display an isolated LPR to bronchial challenge have a positive immediate skin test response to the inciting allergen. Both EPR and LPR can be blocked by premedication with the same agents (49,50).

It is important to note that IgE-mediated mast cell activation contributes to chronic tissue eosinophilia and airway remodeling (51). Tryptase, a major product released into the airway during mast cell activation, stimulates the synthesis of type I collagen by human lung fibroblasts (52). Both histamine and tryptase have direct effects on fibroblast proliferation and on collagen synthesis (53). Mast cells also have been associated with fibrosis in interstitial lung disease (54). They are abundant in fibrotic tissue, and recent studies suggest that they are an ample source of basic fibroblast growth factor (54).

IV. Therapy with Anti-IgE

Neutralization of IgE is a compelling objective because of its central role in atopic disease (54). It became a realistic clinical goal when monoclonal antibodies directed against IgE were found to suppress allergic reactions in animal studies (55). rhuMAb-E25 (omalizumab), a humanized murine monoclonal antibody directed to the FcεRI-binding domain of human IgE, was selected for trials in patients with allergic disease. rhuMAb-E25 binds to free IgE but not to IgA, IgG, or IgE that is attached to mast cells or basophils. It prevents the binding of IgE to FcεRI and thus averts mast cell degranulation that follows cross-linking of IgE by allergens (55). rhuMAb-E25 reduces free plasma IgE in dose-dependent fashion and causes a marked downregulation of FcεRI on basophils (56).

In early clinical trials, treatment with omalizumab reduced free serum IgE levels by more than 90%, markedly suppressed eosinophilia in the induced sputum and limited both EPR and LPR (57,58). A recent phase III study of patients aged 11–50 years with moderate or severe allergic asthma requiring oral and/or inhaled corticosteroids demonstrated the clinical effectiveness of rhuMab-E25 (59). In this study, patients received recommended asthma therapy and one of three experimental regimens: high-dose omalizumab (5.8 μg/kg of body weight per ng of IgE per mL), low-dose omalizumab (2.5 μg/kg of body weight per ng of IgE per mL), or placebo for 20 weeks. During the first 12 weeks patients received a dose of corticosteroids that optimally controlled their symptoms and spirometry results. During the next 8 weeks the corticosteroids were tapered

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as long as symptoms were suppressed and spirometry remained stable. Mean asthma symptom scores and β-agonist use were significantly lower for high-dose omalizumab than placebo at both 12 and 20 weeks, as were the proportions of patients having a >50% reduction in symptom scores (50% vs. 24%; $p < 0.001$ at 12 weeks and 51% vs. 34%; $p = 0.03$ at 20 weeks). Importantly, more patients in the omalizumab groups were able to reduce or discontinue their use of oral/inhaled corticosteroids than in the placebo group. Omalizumab was well tolerated, with no significant differences in the rate of adverse events between active treatment and placebo.

These findings were extended in a large group of children aged from 6 to 12 years with allergic asthma. In a randomized double-blind placebo-controlled study, the effects of the same humanized monoclonal antibody were evaluated over 28 weeks. Two patients were randomized to omalizumab for every one to placebo. These patients were well controlled on inhaled beclomethasone dipropionate and continued on this therapy throughout the first 16 weeks of evaluation. During the subsequent 12 weeks steroid was withdrawn in a standardized way to assess the potential steroid sparing effect of the drug. The patients who received the monoclonal antibody achieved a greater reduction compared to the control group. All of the actively treated patients were able to reduce their steroid dose by at least 50% compared to 71% of the placebo treated patients ($p = 0.001$). In addition there was a significant reduction in the number of asthma exacerbations requiring systemic steroid or a doubling dose of inhaled steroid (placebo 39% vs. omalizumab 18%; $p < 0.001$). Albuterol use for rescue was reduced in the actively treated patients relative to baseline. In addition, the incidence of adverse events was similar between the two treatment groups. These data suggest that IgE does indeed play an important role in the pathophysiology of asthma and that agents that specifically block its interaction with the FCεRI receptor may be a useful adjunct in the management of the disease (59a).

There are patients for whom currently available medical regimens are ineffective. Persistently uncontrolled asthma is associated with reduced glucocorticoid receptor binding on the inflammatory cells (60); anti-IgE may offer additional hope for these patients. Frequent exacerbations of asthma have been documented in patients who do not adhere to their treatment regimen (61). Indeed, studies show that only one half of the medication prescribed for asthma is ever used (62,63). For other patients, imperfect effort and technique limit the benefit of inhaled medication. Thus, the introduction of an effective therapy administered parenterally at 4-week intervals offers a fresh approach to improve the care of many patients.

Most patients with asthma have specific IgE antibodies to aeroallergens that play a central role in the pathogenesis of their disease. Anti-IgE therapy has been shown to reduce free IgE in circulation, suppress both early- and late-phase asthmatic reactions, improve symptoms, stabilize lung function, and decrease the

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need for corticosteroids. Treatment with the anti-IgE antibody omalizumab is well tolerated. It epitomizes a novel approach to the management of chronic asthma that is both imaginative and achievable.

References

1. Gergen PJ, Turkeltaub PC. The association of individual allergen reactivity with respiratory disease in a national sample: data from the second National Health and Nutrition Examination Survey, 1976–80 (NHANES II). J Allergy Clin Immunol 1992; 90(4 Pt 1):579–588.
2. Yssel H, Abbal C, Pene J, Bousquet J. The role of IgE in asthma. Clin Exp Allergy 1998; 28(suppl 5):104–109; discussion 17–18.
3. Postma DS, Bleecker ER, Amelung PJ, Holroyd KJ, Xu J, Panhuysen CI, et al. Genetic susceptibility to asthma—bronchial hyperresponsiveness coinherited with a major gene for atopy. N Engl J Med 1995; 333(14):894–900.
4. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TA. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989; 83(5):875–882.
5. Hamelmann E, Tadeda K, Oshiba A, Gelfand EW. Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness—a murine model. Allergy 1999; 54(4):297–305.
6. Sherrill DL, Stein R, Halonen M, Holberg CJ, Wright A, Martinez FD. Total serum IgE and its association with asthma symptoms and allergic sensitization among children. J Allergy Clin Immunol 1999; 104(1):28–36.
7. Martinez FD. Recognizing early asthma. Allergy 1999; 54(suppl 49):24–28.
8. Stick SM, Arnott J, Turner DJ, Young S, Landau LI, Lesouef PN. Bronchial responsiveness and lung function in recurrently wheezy infants. Am Rev Respir Dis 1991; 144(5):1012–1015.
9. Park ES, Golding J, Carswell F, Stewart-Brown S. Preschool wheezing and prognosis at 10. Arch Dis Child 1986; 61(7):642–646.
10. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995; 332(3):133–138.
11. Cochran D. Diagnosing and treating chesty infants. A short trial of inhaled corticosteroid is probably the best approach. Br Med J 1998; 316(7144):1546–1547.
12. Gern JE, Lemanske RF, Jr., Busse WW. Early life origins of asthma. J Clin Invest 1999; 104(7):837–843.
13. Erb KJ. Atopic disorders: a default pathway in the absence of infection? Immunol Today 1999; 20(7):317–322.
14. Armitage RJ, Goff LK. Functional interaction between B cell subpopulations defined by CD23 expression. Eur J Immunol 1988; 18(11):1753–1760.
15. Jelinek DF. Regulation of B lymphocyte differentiation. Ann Allergy Asthma Immunol 2000; 84(4):375–385.

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16. Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, et al. Surveillance for asthma—United States, 1960–1995. MMWR CDC Surveill Summ 1998; 47(1):1–27.
17. Keeley DJ, Neill P, Gallivan S. Comparison of the prevalence of reversible airways obstruction in rural and urban Zimbabwean children. Thorax 1991; 46(8):549–553.
18. Strachan DP. Lifestyle and atopy. Lancet 1999; 353(9163):1457–1458.
19. Strachan DP, Harkins LS, Johnston ID, Anderson HR. Childhood antecedents of allergic sensitization in young British adults. J Allergy Clin Immunol 1997; 99(1 Pt 1): 6–12.
20. Addo Yobo EO, Custovic A, Taggart SC, Asafo-Agyei AP, Woodcock A. Exercise induced bronchospasm in Ghana: differences in prevalence between urban and rural schoolchildren. Thorax 1997; 52(2):161–165.
21. Strachan DP. Allergy and family size: a riddle worth solving. Clin Exp Allergy 1997; 27(3):235–236.
22. Kramer U, Heinrich J, Wjst M, Wichmann HE. Age of entry to day nursery and allergy in later childhood. Lancet 1999; 353(9151):450–454.
23. Taussig LM, Wright AL, Morgan WJ, Harrison HR, Ray CG. The Tucson Children's Respiratory Study. I. Design and implementation of a prospective study of acute and chronic respiratory illness in children. Am J Epidemiol 1989; 129(6):1219–1231.
24. Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999; 354(9178):541–545.
25. Martinez FD, Stern DA, Wright AL, Taussig LM, Halonen M. Differential immune responses to acute lower respiratory illness in early life and subsequent development of persistent wheezing and asthma. J Allergy Clin Immunol 1998; 102(6 Pt 1):915–920.
26. Ehlenfield DR, Cameron K, Welliver RC. Eosinophilia at the time of respiratory syncytial virus bronchiolitis predicts childhood reactive airway disease. Pediatrics 2000; 105(1 Pt 1):79–83.
27. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. N Engl J Med 1990; 323(8):502–507.
28. Platts-Mills T. Major risk factors according to age: the relevance of indoor allergens to the increase in asthma. In: CE Baena-Cagnani, L Fabbri, S Holgate, PO O'Byrne, eds. Asthma: A Link between Environment, Immunology and the Airways. Seattle: Hogrefe & Huber Publishers, 1999; pp 89–95.
29. Call RS, Smith TF, Morris E, Chapman MD, Platts-Mills TA. Risk factors for asthma in inner city children. J Pediatr 1992; 121(6):862–866.
30. Gelber LE, Seltzer LH, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TA. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. Am Rev Respir Dis 1993; 147(3):573–578.
31. Nelson HS, Szefler SJ, Jacobs J, Huss K, Shapiro G, Sternberg AL. The relationships among environmental allergen sensitization, allergen exposure, pulmonary function, and bronchial hyperresponsiveness in the Childhood Asthma Management Program. J Allergy Clin Immunol 1999; 104(4 Pt 1):775–785.

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32. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989; 320(5):271–277.
33. Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 1997; 336(19):1356–1363.
34. Bryant DH, Burns MW. Skin-prick test reactions to inhalant allergens in asthmatic patients. Med J Aust 1976; 1(24):918–924.
35. Peat JK, Britton WJ, Salome CM, Woolcock AJ. Bronchial hyperresponsiveness in two populations of Australian schoolchildren. III. Effect of exposure to environmental allergens. Clin Allergy 1987; 17(4):291–300.
36. Gerritsen J, Koeter GH, de Monchy JG, Knol K. Allergy in subjects with asthma from childhood to adulthood. J Allergy Clin Immunol 1990; 85(1 Pt 1):116–125.
37. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. Clin Allergy 1977; 7(6):503–513.
38. Van Metre TE, Jr., Marsh DG, Adkinson NF, Jr., Fish JE, Kagey-Sobotka A, Norman PS, et al. Dose of cat (Felis domesticus) allergen 1 (Fel d 1) that induces asthma. J Allergy Clin Immunol 1986; 78(1 Pt 1):62–75.
39. Kniest FM, Young E, Van Praag MC, Vos H, Kort HS, Koers WJ, et al. Clinical evaluation of a double-blind dust-mite avoidance trial with mite-allergic rhinitic patients. Clin Exp Allergy 1991; 21(1):39–47.
40. Dietemann A, Bessot JC, Hoyet C, Ott M, Verot A, Pauli G. A double-blind, placebo controlled trial of solidified benzyl benzoate applied in dwellings of asthmatic patients sensitive to mites: clinical efficacy and effect on mite allergens. J Allergy Clin Immunol 1993; 91(3):738–746.
41. Peroni DG, Boner AL, Vallone G, Antolini I, Warner JO. Effective allergen avoidance at high altitude reduces allergen-induced bronchial hyperresponsiveness. Am J Respir Crit Care Med 1994; 149(6):1442–1446.
42. Piacentini GL, Bodini A, Costella S, Vicentini L, Peroni D, Zanolla L, et al. Allergen avoidance is associated with a fall in exhaled nitric oxide in asthmatic children. J Allergy Clin Immunol 1999; 104(6):1323–1324.
43. Schwartz J, Weiss S. Relationship of skin test reactivity to decrements in pulmonary function in children with asthma or frequent wheezing. Am J Respir Crit Care Med 1995; 152:2176–2180.
44. Spector SL. Leukotriene activity modulation in asthma. Drugs 1997; 54(3):369–384.
45. Determinants of bronchial responsiveness in the European Community Respiratory Health Survey in Italy: evidence of an independent role of atopy, total serum IgE levels, and asthma symptoms. Allergy 1998; 53(7):673–681.
46. Lemanske R, Kaliner M. Late phase allergic reactions. In: Middleton E, Reed C, Ellis E, Adkinson N, Yunginger J, Busse W, eds. Allergy: Principles and Practice. St Louis: Mosby, 1993; pp 320–361.
47. Holgate ST. The inflammatory basis of asthma and its implications for drug treatment. Clin Exp Allergy 1996; 26(suppl 4):1–4.
48. Oettgen HC, Geha RS. IgE in asthma and atopy: cellular and molecular connections. J Clin Invest 1999; 104(7):829–835.
49. Roquet A, Dahlen B, Kumlin M, Ihre E, Anstren G, Binks S, et al. Combined antago-

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nism of leukotrienes and histamine produces predominant inhibition of allergen-induced early and late phase airway obstruction in asthmatics. Am J Respir Crit Care Med 1997; 155(6):1856–1863.
50. Mapp C, Boschetto P, dal Vecchio L, Crescioli S, de Marzo N, Paleari D, et al. Protective effect of antiasthma drugs on late asthmatic reactions and increased airway responsiveness induced by toluene diisocyanate in sensitized subjects. Am Rev Respir Dis 1987; 136(6):1403–1407.
51. Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. J Allergy Clin Immunol 2000; 105(5):847–859.
52. Cairns JA, Walls AF. Mast cell tryptase stimulates the synthesis of type I collagen in human lung fibroblasts. J Clin Invest 1997; 99(6):1313–1321.
53. Levi-Schaffer F, Rubinchik E. Activated mast cells are fibrogenic for 3T3 fibroblasts. J Invest Dermatol 1995; 104(6):999–1003.
54. Inoue Y, King TE, Jr., Tinkle SS, Dockstader K, Newman LS. Human mast cell basic fibroblast growth factor in pulmonary fibrotic disorders. Am J Pathol 1996; 149(6):2037–2054.
55. Shields RL, Whether WR, Zioncheck K, O'Connell L, Fendly B, Presta LG, et al. Inhibition of allergic reactions with antibodies to IgE. Int Arch Allergy Immunol 1995; 107(1–3):308–312.
56. MacGlashan DW, Jr., Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, et al. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol 1997; 158(3):1438–1445.
57. Boulet LP, Chapman KR, Cote J, Kalra S, Bhagat R, Swystun VA, et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. Am J Respir Crit Care Med 1997; 155(6):1835–1840.
58. Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med 1997; 155(6):1828–1834.
59. Milgrom H, Fick RB, Jr., Su JQ, Reimann JD, Bush RK, Watrous ML, et al. Treatment of allergic asthma with monoclonal anti-IgE antibody. rhuMAb-E25 Study Group. N Engl J Med 1999; 341(26):1966–1973.
59a. Milgrom H, Berger W, Naya K, Gupta N, Pollard S, McAlary M, Taylor Fowler A, Rohare P. Treatment of childhood asthma with anti-immunoglobulin E antibody (omalizumab). Pediatrics 2001; 108(2):236.
60. Sher ER, Leung DY, Surs W, Kam JC, Zieg G, Kamada AK, et al. Steroid-resistant asthma. Cellular mechanisms contributing to inadequate response to glucocorticoid therapy. J Clin Invest 1994; 93(1):33–39.
61. Milgrom H, Bender B, Ackerson L, Bowry P, Smith B, Rand C. Noncompliance and treatment failure in children with asthma. J Allergy Clin Immunol 1996; 98(6 Pt 1): 1051–1057.
62. Creer TL, Wigal JK. Self-efficacy [editorial]. Chest 1993; 103(5):1316–1317.
63. Milgrom H, Bender B. Nonadherence to asthma treatment and failure of therapy. Curr Opin Pediatr 1997; 9(6):590–595.
64. Martinez FD. Maturation of immune responses at the beginning of asthma. J Allergy Clin Immunol 1999; 103(3, Pt. 1):355–361.

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13

Anti-inflammatory Activities of Omalizumab (Xolair), A Recombinant Humanized Monoclonal Antibody Binding IgE

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Over a decade of research and clinical efforts in the immunopathogenesis of asthma (1,2) have substantiated the critical role of airway inflammation and identified key mediators and mechanisms. Because information on the pathological features of asthma was first obtained from postmortem examination of severe asthmatics, airway inflammation was believed to be a feature of advanced, terminal disease (3). Those airways showed infiltration by granulocytes (neutrophils, eosinophils), increased numbers of lymphocytes, activated mast cells, collagen deposition beneath the basement membrane, and occlusion of the bronchial lumen by mucus produced from hyperplastic goblet cells. The finding of hypertrophied bronchial smooth muscle supported the widely held belief that asthma was primarily a disease of airway smooth muscle.

The design of a flexible fiberoptic bronchoscope (4) and its application to lung biopsy and, later, lavage (5) made possible the examination of the airways of patients with less severe forms of disease. Studies in the 1980s demonstrated that airway inflammation was a ubiquitous feature (6). These inflammatory changes were observed throughout the central and peripheral (small) airways (7,8). Consequently, asthma is now considered largely an inflammatory disease. International diagnostic and management guidelines (9,10) acknowledge that

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Table 1 Omalizumab Anti-inflammatory Activities

Decrease in	Ref.
IgE, serum	18
Human lung and basophil mediator release	16,21
Mast cell and basophil high-affinity receptor	23
Neutrophils, sputum, and whole blood	40
Monkey and human skin wheal-and-flare reaction	15,47
Methacholine hyperreactivity	26
Nasal inflammatory cells	49
Bronchial hyperreactivity	
Specific (allergen early/late phases)	26
Eosinophils, whole blood	35
Eosinophils, sputum	27
Exhaled nitric oxide	46

asthma results from complex interactions among inflammatory cells, mediators, and resident airway tissues.

An esential effector molecule in this allergic airways inflammation is immunoglobulin-E (IgE). Following the knowledge of the role IgE plays in all Type I hypersensitivity diseases (11), biomedical scientists labored for three decades to block IgE interaction with immune effector cells expressing IgE receptors (12). With the engineering of a humanized recombinant monoclonal antibody (MAb) (13) binding IgE at an epitope identical to the high-affinity receptor (14), those interested in the immunopathogenesis of asthma now have a safe and effective probe with which to delineate the contribution of IgE to various features of asthmatic clinical disease (see Chaps. 11 and 12). In the sections that follow the anti-inflammatory activities of an anti-IgE (Xolair, omalizumab) will be reviewed (Table 1).

I. Anti-inflammatory Effects of Omalizumab in Preclinical Studies

Prerequisite safety (15,16) experiments confirmed the epitope mapping studies, which demonstrated that both omalizumab and the α chain of FcεRI bound the same FcεIII domain epitope. Peripheral blood basophils were obtained from healthy donors, sensitized by the addition of human serum containing ragweed-specific IgE, and histamine release was measured following the addition of ragweed antigen. The addition of omalizumab to sensitized cells failed to induce histamine release, indicating that omalizumab is unable to cross-link cell-bound IgE and trigger mediator release. The inability of omalizumab to bind to basophils

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was confirmed by the finding that the cells showed no positive immunofluorescence staining for omalizumab (15,17).

A. Inhibition of Histamine Release and Contractile Response of Human Lung Tissue

Segments of lung parenchyma obtained from patients undergoing lung surgery were passively sensitized with serum from a ragweed-allergic subject in the presence of an equimolar concentration of omalizumab. Challenge with ragweed antigen induced histamine release and a contractile response, assessed as percent of the maximum contraction induced by KCl. Omalizumab administered at the time of passive sensitization completely blocked both histamine release and tissue contraction on antigen challenge (Fig. 1) (16). In another experiment when the passively sensitized human lung tissue was challenged with omalizumab, there was no detectable histamine release or tissue contraction, confirming that omalizumab does not bind to human IgE that is already bound to its high-affinity receptor on lung cells.

B. Reduction in Skin Responsiveness in Sensitized Cynomolgus Monkeys

The ability of omalizumab to affect IgE responses in vivo was measured in cynomolgus monkeys (15) where the affinity of omalizumab for cynomolgus monkey

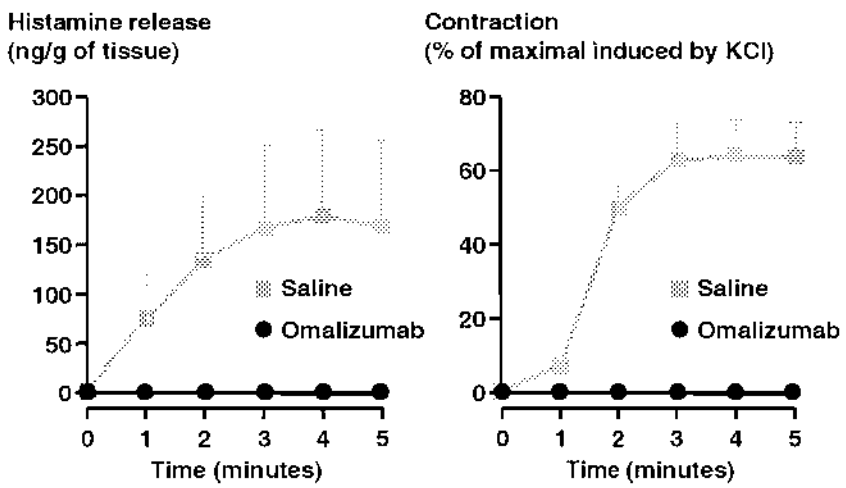

Figure 1 Human lung strips were passively sensitized with human ragweed serum in the presence or absence (saline) of omalizumab in equimolar concentrations to IgE. Omalizumab blocked histamine release (left graph) and tissue contraction (right graph) in response to ragweed challenge.

IgE (3×10^{-10} units) is nearly equivalent to that for human IgE (1.7×10^{-10} units). Injection of omalizumab into the skin failed to elicit the wheal and flare reaction typical of mast cell degranulation, nor did it induce hive formation in monkey skin presensitized with human ragweed-specific IgE. Identical results were obtained when omalizumab was administered systemically. Doses as high as 50 mg/kg did not cause a systemic anaphylactic reaction confirming the safety of an anti-IgE binding free IgE at the same heavy chain site as FcεRI.

Two weeks after a single systemic dose of omalizumab, given at a concentration in excess of serum IgE levels, five out of six monkeys had a reduction in the skin response to ragweed allergen, as demonstrated by an increase in the dose of ragweed required to elicit hive formation. After a second dose of omalizumab given 4 weeks later, all six monkeys evidenced reduced skin responsiveness. The reduced responsiveness persisted at week 10, 4 weeks after the second dose of omalizumab. These results occurred in parallel with a reduction in serum free IgE levels.

II. Anti-inflammatory Effects of Omalizumab in Studies of Human Physiology and Clinical Trials

A. Pharmacodynamic Effect on Serum Free IgE

Serum free levels of IgE fall to the therapeutic target range of 2–50 ng/mL (≤10% of baseline values) within minutes of intravenous administration of omalizumab and within 24 hours of subcutaneous administration (Fig. 2). During ad-

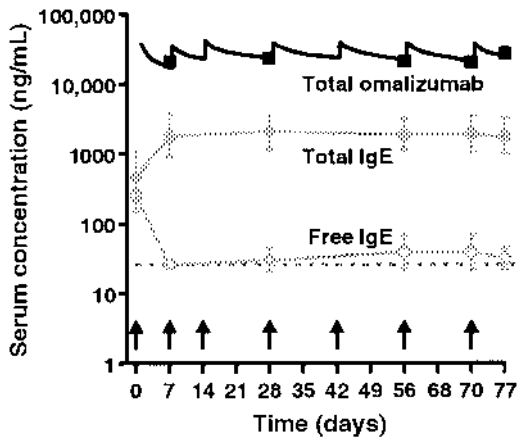

Figure 2 Pharmacodynamic profile from phase I studies showing serum concentrations of omalizumab, total IgE, and free IgE. The arrows indicate dosing events.

ministration there is a four- to sixfold increase in serum levels of total IgE, since the measurement includes IgE that is complexed to omalizumab.

The low serum levels of free IgE are maintained with repeated administration. It has been shown that an adequate suppression of free IgE levels is necessary in order for a clinical effect to become evident. This was demonstrated in an early dose-ranging study in patients with seasonal allergic rhinitis (SAR) (18) demonstrating that those patients whose serum level of free IgE had fallen to the lowest levels achieved lower symptom scores. The study showed that the decrease in free IgE depended on the dose of omalizumab adjusted for the patient's baseline IgE (MAb ligand), so that for any starting concentration of free IgE, higher doses of omalizumab produced a greater decrease in serum free IgE. The necessity for suppression of serum free IgE to very low levels, which can be achieved through a dosing schedule that provides omalizumab in excess (10- or 15-fold) of free serum IgE concentrations and according to an individual patient's baseline level of IgE, was an important advance in pharmacodynamic knowledge with this agent.

B. Effect on IgE Synthesis

An interaction between omalizumab and membrane-bound IgE on B cells can be theorized, since B-cell IgE is anchored to the cell membrane via a peptide extension, leaving the Ig FcεIII site accessible to the anti-IgE molecule. The possibility that anti-IgE antibody treatment would have an effect on IgE synthesis had been suspected for many years since Bozelka et al., in 1982, showed in mice that IgE isotype synthesis by B cells could be inhibited in the long term using polyclonal anti-IgE (19). These findings were confirmed by Haak-Frendscho et al. (20), who observed in adult, ovalbumin-sensitized mice that treatment with a monoclonal anti-IgE antibody inhibited antigen-specific IgE synthesis. Splenocytes from mice were unable to synthesize IgE in vitro. This work was extended by Shields et al. (15), who examined the effect of omalizumab on antigen-specific IgE synthesis by human B cells. Using peripheral blood mononuclear cells (PBMCs) obtained from allergic subjects, they showed that stimulation of IgE synthesis by the addition of cat antigen was suppressed to baseline levels in the presence of omalizumab.

These results in short-term cell culture systems do not appear to translate into an effect on IgE synthesis in humans in vivo. As shown in Figure 2, total serum levels of IgE (comprising IgE-omalizumab complexes) increase and then plateau at steady-state omalizumab. If there was a reduction in newly synthesized IgE, the levels of total serum IgE would be expected to subside over time. This is not the case. In addition, serum levels of free IgE tend to increase with waning omalizumab concentrations towards the end of each interval (2–4 weeks) between doses of omalizumab. This finding indicates continued IgE synthesis. In

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a phase I study (Genentech Protocol Q0673g), subjects with perennial allergic rhinitis were treated with omalizumab using two high doses, 0.015 or 0.030 mg/kg per IU/mL of IgE, every 2 weeks for 26 weeks. At week 28 the dose of omalizumab was reduced to 0.0050 or 0.0015 mg/kg/IU/mL, testing the hypothesis that suprapharmacological doses would suppress IgE synthesis permitting maintenance of low serum free IgE levels by a significantly lower dose of anti-IgE. However, serum free IgE levels rose slowly on this low-dose protocol (synthesis was not suppressed) and by 8 weeks had reached 16% of values at enrollment; at study termination (12 weeks after the last dose of omalizumab) serum free IgE was 62% of enrollment values. The extent of ''front-end loading'' with high doses of omalizumab during the first part of the study (26 weeks) did not affect the degree of suppression of free IgE, suggesting a lack of in vivo effect on ongoing IgE synthesis in atopic subjects. These observations from multiple studies allow one to conclude that inhibition of IgE synthesis is not occurring in humans treated with anti-IgE.

C. Effect on Expression of High- and Low-Affinity Receptors

A fundamental advance in understanding of how omalizumab exerts its clinical effect was obtained by MacGlashan and colleagues (21), who demonstrated that as omalizumab decreased serum IgE, cell-bound IgE fell, basophil high-affinity receptor (FcεRI) expression decreased, and consequently allergen responsiveness of these cells was reduced. They examined the expression of IgE and FcεRI on human basophils taken from 12 allergic subjects who had received intravenous omalizumab for 3 months. In this study serum free IgE levels were reduced to 1% of pretreatment values during omalizumab treatment. Basophil surface receptor IgE densities were significantly reduced from a pretreatment average of 240,000 molecules of IgE per cell to approximately 2200 per cell, a greater than 99% decrease ($p = 0.0022$—a change mirroring precisely the fall in serum IgE. Total FcεRI density, measured using sensitized cells, decreased similarly by approximately 97%, from an average of 240,000 pretreatment to 8600 receptors per cell after treatment ($p = 0.0022$). Combining these results, it can be seen that 3 months of treatment with omalizumab results in approximately threefold more unoccupied than occupied receptors.

Basophils were also assessed for their ability to release the pro-inflammatory mediator histamine in response to various stimuli. Histamine release in response to stimulation with dust mite antigen decreased by 90% ($p = 0.002$), in comparison with an essentially unchanged response in controls. In contrast, the response to FMLP, which acts through a distinct receptor on basophils, was unchanged and following in vitro treatment with a nontherapeutic anti-IgE that cross-links surface IgE histamine release was decreased 40%.

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Studies in both mice (22) and human (23) mast cells parallel the findings discussed above for human peripheral blood basophils. High-affinity receptors (FcεRI) on the surface of mast cells are regulated by ambient concentrations of IgE. IgE is known to modulate the expression of FcεRII on B cells, where IgE appears to stabilize the cell-surface expression of FcεRII by preventing its proteolytic cleavage (24).

D. Effect on Early- and Late-Phase Asthmatic Responses

Deposition of allergen by inhalation or bronchoscopy in allergic asthmatics induces an immediate or early-phase asthmatic response (EAR) within minutes, involving acute bronchoconstriction that is attributable largely to histamine released by mast cells (2,25). In 50–60% of subjects this is followed 2 hours later by a second, prolonged phase of bronchoconstriction known as the late-phase asthmatic response (LAR), attributable to the influx and activation of inflammatory cells, notably the eosinophil and the T lymphocyte. Since these events may model the acute and chronic inflammatory disease processes of asthma, the effect of pharmacological treatment upon the allergen-induced EAR and especially the highly cellular LAR is of interest.

Boulet et al. (26), in a study of 19 patients with mild allergic asthma, reported that omalizumab significantly reduced the allergen-induced EAR, assessed as an increase in the provocative concentration of allergen required to produce a 15% fall in FEV_1 (PC_{15}). Significant increases of 2.3, 2.2, and 2.7 doubling concentrations of allergen were observed as early as day 27 of treatment and also at later times (55 and 77 days of treatment, respectively) (compared with -0.3, 0.1, and -0.8 with placebo; $p \leq 0.002$). There was a trend towards greater increases in doubling concentrations in subjects with the greatest reduction in serum free IgE with optimal EAR effects at serum free IgE $\leq$ 24 ng/mL.

In a separate study in 19 subjects with mild allergic asthma, omalizumab was found to blunt the LAR as well as EAR following inhalation of allergen (27). The protocol allowed up to one greater doubling dose of allergen to be given for the second challenge after 9 weeks of treatment. The maximal percentage fall in FEV_1 during the EAR reduced 42% with omalizumab ($p < 0.02$) despite 7/9 subjects receiving more Ag; importantly the LAR fall in FEV_1 was reduced 65% ($p < 0.02$). The improvement in LAR following omalizumab treatment in this study (27) could be viewed as predicting future efficacy of this anti-IgE in significantly reducing asthmatic exacerbations, for studies over the past 20 years have suggested that the occurrence of late responses following allergen challenge are relevant to the clinical expression and severity of allergic asthma (28). For example, the development of LAR has been associated with more severe clinical asthma as determined by frequency of asthma attacks in the preceding year (29).

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E. Effect on Nonspecific Bronchial Hyperresponsiveness

Bronchial hyperresponsiveness (BHR) to nonspecific stimuli is a cardinal feature of asthma and has been shown to relate to the severity of disease (30). BHR is generally regarded as a consequence of the chronic airways inflammation of asthma. Airway sensitization, mediated by IgE, appears to be closely related to nonspecific BHR, perhaps through an indirect effect of pro-inflammatory cytokines and mediators (31,32).

Boulet et al.'s study of the effect of omalizumab on the allergen-induced early-phase response also included an assessment of the effect on associated nonspecific BHR measured by methacholine challenge (26). Mean methacholine PC_{20} values increased from 0.73 to 1.34 mg/mL after 11 weeks of treatment, a change of 0.9 doubling concentrations ($p < 0.05$ compared with a decrease [increased sensitivity] in the placebo group). Methacholine PC_{20} was also measured in the study by Fahy's group (27). The values 24 hours after allergen challenge were reduced significantly during treatment (from 0.13 to 0.45 mg/mL, $p < 0.02$ compared with the increased sensitivity in the placebo group from 0.49 to 0.29 mg/mL).

F. Effects on Eosinophils

Eosinophils are a highly recognizable cell in the inflammatory process of asthma (33). Eosinophilopoiesis begins in the bone marrow, is regulated by IL-3, IL-5, and GM-CSF, and IL-5 induces terminal differentiation of immature eosinophils. They are mobilized from bone marrow under the influence of IL-5 and eotaxin and are selectively recruited to the airways via upregulated expression of appropriate adhesion molecules (e.g., ICAM1, VCAM1) on vascular endothelial cells. Eosinophils are primed and activated in the airways tissue by cytokines (such as IL-5) and chemokines (such as eotaxin, MIP-1a, MCP1, and RANTES). Mast cell–derived cytokines may be involved in the stimulation of eotaxin production by epithelial and endothelial cells. Once activated, eosinophils release granule proteins (MBP, peroxidase, eosinophil-derived neurotoxin) that are highly cytotoxic, especially for epithelial cells, as well as the release of mediators and cytokines that further exacerbate the inflammatory process. Rajakulasingam et al. (34) reported that 80–90% of cells recruited 24 hours after allergen challenge were eosinophils, and these cells formed the majority (85–95%) of FcεRI+ cells (in contrast to the findings in stable asthma, where eosinophils do not constitutively express the receptor). These results support a major role for IgE-dependent mechanisms in the increased asthma symptoms and disease flares induced by allergen exposure.

In the study by Fahy et al. (27), the percentage of eosinophils in induced sputum was measured before and after allergen challenge during treatment with

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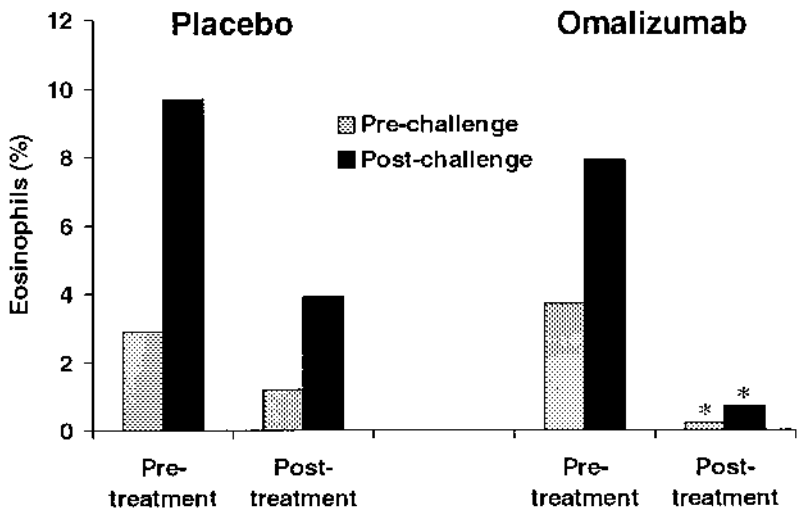

Figure 3 Median percentage of eosinophils in induced sputum before and 24 hours after allergen challenge at baseline and at the end of 9 weeks of treatment with placebo (n = 7) or omalizumab (n = 8) in subjects with mild allergic asthma. *$p < 0.05$ vs. pretreatment values; not significantly different from the corresponding changes in the placebo group.

omalizumab. The percentage of eosinophils after allergen challenge was reduced 11-fold, a significant change from baseline values (Fig. 3).

Blood eosinophil percentages were assessed in the same bronchial allergen challenge study. The changes mirrored those seen in induced sputum. Blood eosinophil percentages decreased significantly with omalizumab treatment (from 4.6 ± 0.8% to 2.4 ± 0.3%; $p = 0.008$), but this decrease was not statistically different from the change in the placebo group (4.4 ± 0.7% to 3.9 ± 0.7%).

The results from a subset of patients evaluated in a phase III clinical study in adults with moderate-to-severe asthma provided further evidence of a positive trend to a biological effect (Table 2) (35). In 513 patients evaluated, the circulating eosinophil counts were reduced in patients treated with omalizumab, and fewer patients had abnormally elevated eosinophil counts. These positive trends were also evident during the second phase of the study at a time when patients had reduced their dose of inhaled corticosteroid (ICS) under cover of omalizumab treatment (overall results showed a median percent reduction in ICS dose of 75% vs. 50% on placebo, and 40% of omalizumab-treated patients vs. 19% of placebo patients withdrew from glucocorticoid treatment completely, both $p <$ 0.001) (36).

Table 2 Effect of Omalizumab on Blood Eosinophilia in Patients with Moderate-to-Severe Allergic Asthma

Treatment	Baseline	End of add-on phase[a]	End of steroid -reduction phase
Absolute eosinophil counts ($\times 10^7$/L)[b]			
Omalizumab	31 (26)	−6.0 (−3.0)	−2.0 (−3.0)
Placebo	33 (29)	−1.0 (0.0)	+2.0 (+1.0)
Patients with abnormally elevated eosinophil counts[c]			
Omalizumab	14.5%	5.7%	9.7%
Placebo	13.9%	12.6%	18.3%

[a] Omalizumab was added to existing inhaled corticosteroid (ICS) treatment, followed by a phase in which the ICS dose was tapered.
[b] Mean (median) value at baseline and changes during the study.
[c] Percentage of patients with counts $>50 \times 10^7$/L.

G. Effect on Neutrophils

Several reports have suggested that neutrophils are prominent in the airways of patients with acute severe asthma. They may also be involved in the pathogenesis of severe persistent asthma, which could help explain the corticosteroid insensitivity that can occur in such patients (37). Neutrophil recruitment and activation appear to be influenced by IL-8 (38), possibly arising from epithelial cells or as an autocrine loop. Neutrophils are important sources of cytokines and proteolytic enzymes such as myeloperoxidase (MPO), with implications for direct asthmatic tissue injury and airway remodeling.

A subset of patients with moderate-to-severe allergic asthma from a phase IIb study of omalizumab (39) were assessed for the effect of anti-IgE treatment on neutrophil markers in induced sputum (40). Omalizumab was added to existing treatment with inhaled or oral corticosteroids, and induced sputum samples were collected before and after 12 weeks of treatment. Fourteen patients received high or low doses of omalizumab; 11 received placebo. Treatment with omalizumab was associated with a decrease in the proportion of neutrophils from 41% at baseline to 32% at 12 weeks; in the placebo group neutrophils increased by 4.4%. MPO concentrations were unchanged in the placebo group and decreased from 2.6 μg/mL at baseline to 1.1 μg/mL after omalizumab treatment. While the changes were not statistically significant in this small group of patients, the results suggest that omalizumab treatment might be associated with a reduction in numbers and markers of neutrophilic inflammation. If confirmed, this would be an interesting distinction for omalizumab from the mode of action of corticosteroids, which prolong neutrophil survival by inhibiting apoptosis.

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H. Effect on Exhaled NO in Children

Exhaled nitric oxide (eNO) has been considered as a marker of airway inflammation in asthma (41,42). Levels of eNO are elevated in steroid-naive asthmatic subjects compared with normal subjects and decrease with corticosteroid treatment (43). A study in children found that eNO was elevated in atopic asthmatics compared with nonatopics, correlated with serum IgE, and children with nonatopic asthma had levels similar to those of nonasthmatics (44). Extending these findings, Simpson (45) reported that eNO was elevated in patients with atopic asthma who were exposed to their relevant allergen compared with sensitized patients who were not exposed.

Exhaled NO levels were studied in a subset of patients from a phase III clinical study of omalizumab (Protocol 010) in children 6–12 years of age with mild-moderate allergic asthma. All patients were receiving ICS treatment and were asymptomatic on study entry. Omalizumab or placebo was added to existing therapy in the first 16-week phase of the study. This was followed by a 12-week phase in which the dose of ICS was gradually reduced. After this 7-month double-blind treatment period, active treatment was continued for an additional 5 months in an open-label fashion available to patients in both groups. The eNO subset at two participating centers comprised 18 patients receiving omalizumab and 11 receiving placebo.

The sample size was small and the variability high. Nevertheless, statistical differences between active and placebo groups were observed at several time-points during the steroid-reduction phase (46), supporting the notion that omalizumab prevents an increase in airway inflammation at a time when ICS treatment is reduced or withdrawn completely (Fig. 4). It is interesting to note the significant decline in eNO levels in patients who were switched from placebo to omalizumab for the follow-up (Table 3). These preliminary findings strongly support an anti-inflammatory activity of omalizumab in allergic asthma.

I. Effect on Human Skin Responsiveness to Allergen Challenge

The effect of omalizumab on skin responsiveness to allergen challenge has been reported in several studies. This is of interest as an indicator of systemic anti-inflammatory activity.

In a phase I open-label study reported by Togias et al. (47), omalizumab was administered intravenously to 47 subjects with perennial allergic rhinitis at doses of 0.015 and 0.03 mg/kg per IU/mL of IgE given every 2 weeks. These doses were continued for 26 weeks. Patients were then rerandomized to receive one of two lower doses (0.0015 or 0.005 mg/kg/IU/mL), which were continued from week 27 to 54 weeks. The skin prick test response to dust mite antigen (*Dermatophagoides farinae* or *Dermatophagoides pteronyssinus*) was assessed

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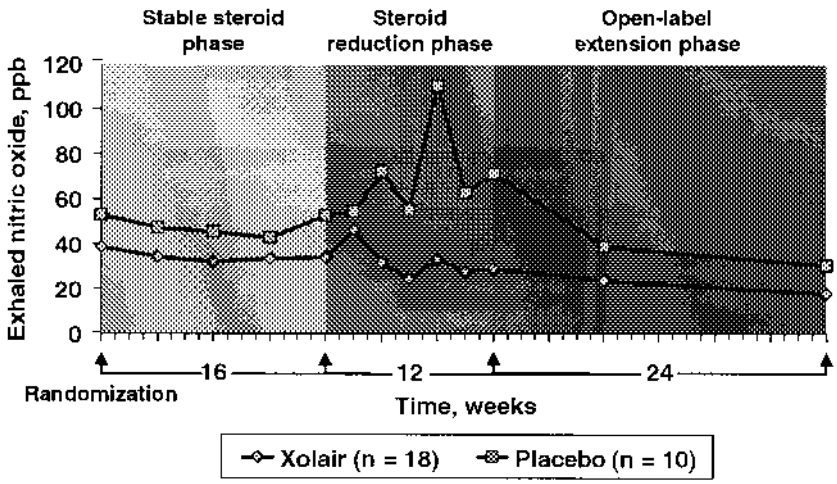

Figure 4 Xolair reduces exhaled nitric oxide, a marker of airway inflammation. Exhaled NO levels studied in 6- to 12-year-old asthmatics were controlled on inhaled CS and rescue β-agonist use. During a 12-week protocol-defined withdrawal of CS, eNO levels increased in those children receiving placebo. Omalizumab-treated patients were protected against increased eNO while achieving significantly greater withdrawal of CS.

at screening, twice during the initial dose phase (at weeks 14 and 26) and at various times during the reduced dosage period.

Wheal area was significantly suppressed at both testing points during the initial high-dose phase, with an average decrease in wheal area of 73% and 72% at weeks 14 and 26. The changes were similar and significantly decreased from

Table 3 Levels of eNO (MEAN ± SD, ppb)

	Omalizumab	Placebo
Baseline	38.6 ± 25.6	52.7 ± 52.0
16 weeks (end of add-on phase)	33.9 ± 19.0	52.5 ± 27.8
28 weeks (end of steroid-reduction phase)	29.1 ± 20.5[a]	71.1 ± 50.5
52 weeks (end of open-label follow-up)	18.0 ± 21.8[b]	30.2 ± 21.9[c] (patients switched to active treatment)

[a] $p < 0.05$ vs. corresponding value in placebo group.
[b] $p < 0.05$ vs. baseline value.
[c] $p < 0.05$ vs. value at end of steroid-reduction phase.

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baseline at both initial doses. The lower doses given in the second dosage phase (after 26 weeks) were ineffective in suppressing dermatological inflammation as wheal areas returned to baseline values following increased serum IgE.

Similar results were observed in a subgroup of 12 patients from the same study, separately reported (21). After a 3-month treatment period, skin prick tests to dust mite antigen required approximately 100-fold more antigen for a wheal-and-flare reaction equal to that measured before treatment ($p = 0.0007$).

III. Anti-inflammatory Effects in the Upper Airway

It is likely that IgE contributes to the allergic inflammation apparent in the nose and conjunctiva of patients suffering with rhinoconjunctivitis. B cells in the nasal mucosa of patients with hay fever express IgE and allergen-induced heavy-chain switching to IgE occurs locally (48). Anti-IgE (E25) was administered at two dose levels in an open label safety study to 20 subjects with perennial allergic rhinitis (PAR) and a positive skin prick test to dust mites (49). Nasal lavage fluids were obtained before and 4 days after provocative nasal antigen challenge with dust mite allergen performed at baseline screening period and days 84 and 168 of E25 treatment. Serum level of free IgE were suppressed to $\leq$15 ng/mL during the study in both treatment groups. Nasal allergen challenge symptom scores decreased substantially (Table 4). Symptom decreases were largest in the sneezing and rhinorrhea domains. E25 treatment through day 84 and 168 blocked post-challenge rise in IgE plaque-forming cells (decreased by $>$50% and mite-specific IgE decreased by $>$25%). This was the first demonstration that PAR treated with anti-IgE caused a marked reduction in nasal reactivity to allergen and a reduction in both IgE-producing B lymphocytes and mite-specific IgE levels.

Phase III studies in allergic asthma provide the most clinically relevant results since omalizumab was administered subcutaneously at current doses to large numbers of patients with moderate-severe asthma. These more recent stud-

Table 4 Symptom Scores in PAR Patients Treated with Omalizumab Following Nasal Challenge

Part I group	*n*	Baseline	Day 84, *p*-value	Day 168, *p*-value
0.015 mg/kg/IU/mL	10	5.40 ± 1.07	1.80 ± 1.32, $p = 0.002$	2.10 + 2.74, $p = 0.008$
0.030 mg/kg/IU/mL	10	5.00 ± 1.15	2.00 ± 2.11, $p = 0.006$	1.67 ± 1.00, $p = 0.004$

Displayed values are mean ± SD. *p*-values are from Wilcoxon single rank test.

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ies confirm that omalizumab can partially or totally substitute for inhaled corticosteroids in the long term (1 year) without deterioration of asthma control, providing a useful, relevant, albeit indirect marker of anti-inflammatory activity.

In all three of the pivotal phase III studies, omalizumab was significantly superior to placebo with respect to the percentage reduction in inhaled corticosteroid dose attained and the proportion of patients who achieved steroid reduction or withdrawal.

IV. Conclusion

It has been established that IgE plays a pivotal role in the cascade of biochemical events leading to allergic inflammation (Fig. 5). IgE antibodies are central mediators of the inflammatory reactions responsible for induction and maintenance of allergic symptoms. The potential for IgE to mediate inflammation in the airway is supported by studies demonstrating allergen-specific IgE synthesis in the lung (50,51) and human airway cells express high- or low-affinity receptors for IgE (52,53). When cell-bound IgE on lung cells is cross-linked by an antigen, the cell degranulates releasing mediators (histamine, leukotrines, and PAF-4) (11,54,55).

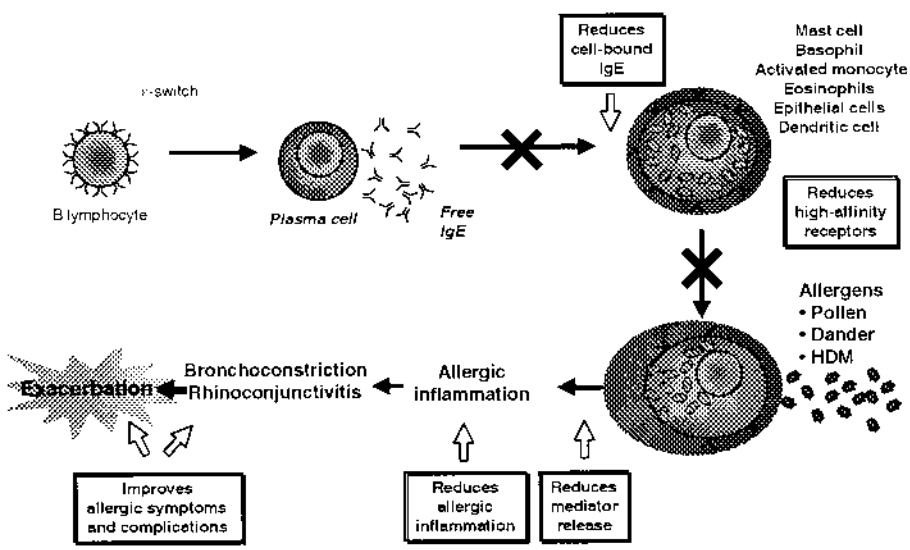

Figure 5 Xolair (omalizumab) mechanism of action in the allergic inflammatory cascade.

These inflammatory mediators are responsible for the swelling, erythema, and smooth muscle contraction characteristic of allergic inflammation. Indeed, as detailed in this chapter, a therapy designed to bind IgE and decrease free IgE levels ameliorates airways inflammation (e.g., eosinophils, neutrophilia), decreases markers of airway inflammation (e.g., eNO), and physiological consequences of inflammation (PC_{20} meth, bronchoprovocation LAR). Allergic clinical disease of the upper and lower airways would be predicted to be significantly improved by anti-IgE therapy (Xolair, omalizumab).

References

1. Vignoal AM, Gagliardo R, Guerrera D, Chiappara G, Chanez P, Bousquet J, Bonsignore G. New evidence of inflammation in asthma (review). Thorax 2000; 55(suppl 2):S59-60.
2. Fick RB, Metzger WJ, Richardson HB, Zavala DC, Moseley PL, Schoderbek WE, Hunninghake GW. Increased bronchovascular permeability following allergen exposure in asthmatics. J Appl Phys 1987; 63(3):1147–1155.
3. Dunnill M. The pathology of asthma, with special reference to changes in the bronchial mucosa. J Clin Pathol 1960; 13:27–33.
4. Ikeda S, Yanai N, Ishikawa S. Flexible bronchofiberscope. Keio J Med 1968; 17:1–16.
5. Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. J Lab Clin Med 1974; 84:559–573.
6. Rankin JA, Snyder Pe, Schachter EN, Matthay RA. Bronchoalveolar lavage—its safety in mild asthma. Chest 1984; 85:723–728.
7. Haley KJ, Sunday ME, Wiggs BR, Kozakewich HP, Reilly JJ, Mentzer SJ, Sugarbaker DJ, Doerschuk CM, Drazen JM. Inflammatory cell distribution within and along asthmatic airways. Am J Respir Crit Car Med 1998; 158:565–572.
8. Kraft M, Djukanovic R, Wilson S, Holgate ST, Martin RJ. Alveolar tissue inflammation in asthma. Am J Respir Crit Care Med 1996; 154:1505–1510.
9. Bousquet J. Global initiative for asthma (GINA) and its objectives. Clin Exp Allergy 2000; 30(suppl)1:2–5.
10. National Institutes of Health, National Heart, Lung and Blood Institute. Highlights of the Expert Panel Report II. Guidelines for the Diagnosis and Management of Asthma, Bethesda, MD: National Institutes of Health, 1997. NIH Publication 97-4051A.
11. Ishizaka K, Ishizaka T, Lee EH. Biologic function of the Fc fragments of E myeloma protein. Immunochemistry 1970; 7:687.
12. Heusser C, Jardieu P. Therapeutic potential of anti-IgE antibodies. Rev Curr Opin Immunol 1997; 9/6.
13. Presta L, Lahr S, Shields R, Porter J, Gorman C, Fendly B, Jardieu P. Humanization of an antibody directed against immunoglobulin E. J Immunol 1993; 151(5):2623–2632.
14. Presta L, Shields R, O'Connell L, Lahr S, Porter J, Gormen C, Jardieu P. The binding

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site on human immunoglobulin E for its high affinity receptor. J Biol Chem 1994; 269(42):26368–26373.
15. Shields R, Werther W, Zioncheck K, O'Connell L, Fendly B, Presta L, Thomas D, Saban R, Jardieu P. Inhibition of allergic reactions with antibodies to IgE. In: Galli S, Sheffer D, eds. Proceedings 20th Symposium Collegium Internationale Allergologicum, 1995; 107(103):308–312.
16. Saban R, Haak-Frendscho M, Zine M, Ridgway J, Gorman C, Presta LG, Bjorling D, Saban M, Jardieu P. Human FcεRI-IgG and humanized anti-IgE monoclonal antibody MaE11 block passive sensitization of human and rhesus monkey lung. J Allergy Clin Immunol 1994; 94(5):836–843.
17. Fei DTW, Lowe J, Jardieu P. A novel bioactivity assay for monoclonal antibodies directed against IgE. J Immunol Methods 1994; 171:189–199.
18. Casale TB, Bernstein IL, Busse WW, LaForce Cr, Tinkelman DG, Stoltz RR, Dockhorn RJ, Reimann J, Su JQ, Fick RB, Adelman DC. Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. J Allergy Clin Immunol 1997; 100:110–121.
19. Bozelka E, McCants ML, Salvaggio JE, Lehrer SB. IgE isotype suppression in anti-ε-treated mice. Immunology 1982; 46:527–532.
20. Haak-Frendscho M, Robbins K, Lyon R, Shields R, Hooley J, Schoenhoff M, Jardieu P. Administration of an anti-IgE antibody inhibits CD23 expression and IgE production in vivo. Immunology 1994; 82:306–312.
21. MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, Mackenzie-White J, Sterbinsky SA, Hamilton RG, Lichtenstein LM. Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol 1997; 158(3):1438–1445.
22. Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I, Kinet JP, Galli S. IgE enhances mouse mast cell FcεRI expression in vitro and in vivo. Evidence for a novel amplification mechanism in IgE-dependent reactions. J Exp Med 1997; 185:663–672.
23. Yano K, Yamaguchi M, de Mora F, Lantz CS, Butterfield JH, Costa JJ, Galli SJ. Production of macrophage inflammatory protein-1α by human mast cells. Increased anti-IgE-dependent secretion after IgE-dependent enhancement of mast cell IgE binding ability. Lab Invest 1997; 77(2):185–193.
24. Lee WT, Rao M, Conrad DH. The murine lymphocyte receptor for IgE. The mechanism of ligand specific receptor upregulation on B cells. J Immunol 1987; 139:1191–1198.
25. Fick RB, Richardson HB, Zavala DC, Hunninghake GW. Bronchoalveolar lavage in allergic asthmatics. Am Rev Respir Dis 1987; 135(5):1204–1209.
26. Boulet L-P, Chapman KR, Cote J, Kalra S, Bhagat R, Swystun V, Laviolette M, Cleland LD, Deschesnes F, Su JQ, DeVault A, Fick RB, Cockcroft DW. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. Am J Respir Crit Car Med 1997; 155:1835–1840.
27. Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, Fick RB, Boushey HA. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med 1997; 155:1828–1834.

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28. Durham SR. The significance of late responses in asthma. Clin Exp Allergy 1991; 21:3–7.
29. Warner JO. Significance of late reactions after bronchial challenge with house dust mite. Arch Dis Child 1976; 51:905–910.
30. Fowler SJ, Dempsey OJ, Sims EJ, Lipworth BJ. Screening for bronchial hyperresponsiveness using methacholine and adenosine monophosphate. Relationship to asthma severity and $beta_2$-receptor genotype. Am J Respir Crit Care Med 2000; 162: 1318–1322.
31. Tunon de Lara JM. Sensitization of human airways: What is the role of immunoglobulin-E? Clin Exp Allergy 1998; 28:660–663.
32. Schmidt D, Watson N, Ruehlmann E, Magnussen H, Rabe KF. Serum immunoglobulin E levels predict human airway reactivity in vitro. Clin Exp Allergy 2000; 30: 233–241.
33. Gleich GJ. Mechanisms of eosinophil-associated inflammation. J Allergy Clin Immunol 2000; 105:651–663.
34. Rajakulasingam K, Till S, Ying S, Humbert M, Barkans J, Sullivan M, Meng Q, Corrigan CJ, Rungre J, Grant JA, Kay AB, Durham SR. Increased expression of high affinity IgE (FcεRI) receptor-alpha chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma. Am J Respir Crit Care Med 1998; 158:233–240.
35. RB Fick, PW Rohane, N Gupta, M Marian, J Cooper, M McAlary, E Gibson, M Ashby. Anti-inflammatory effects of a recombinant monoclonal anti-IgE (E25) in asthma. Am J Respir Crit Care Med 2000; 161(3):A199.
36. Busse WW, Corren J, Lanier BQ, Lee T, Nayak A, Chervinsky P, Gibson E, Fick R, Fowler-Taylor A, Gupta N, Rohane PW. rhuMAb-E25 (E25), a novel therapy for the treatment of allergic asthma. J Allergy Clin Immunol 2000, 105(6, pt 1):5.
37. Wenzel SE, Szefler SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. Am J Respir Crit Care Med 1997; 156:737–743.
38. Norzila MZ, Fakes K, Henry RL, Simpson J, Gibson PG. Interleukin-8 secretion and neutrophil recruitment accompanies induced sputum eosinophil activation in children with acute asthma. Am J Respir Crit Car Med 2000; 161:769–774.
39. Milgrom H, Fick RB, Jr, Su JQ, Reimann JD, Bush RK, Watrous ML, Metzger WJ, for the rhuMAb-E25 Study Group. Treatment of allergic asthma with monoclonal anti-IgE antibody. N Engl J Med 1999; 341:1966–1973.
40. Busse WW, Fahy JV, Fick. A pilot study of the effects of an anti-IgE antibody (E25) on airway inflammation in moderate-severe asthma. Am J Respir Crit Care Med 1998; 157(3):A456.
41. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. Lancet 1994; 343: 133–135.
42. Gaston B, Drazen JM, Chee CB, Wohl ME, Stamler JS. Expired nitric oxide (NO) concentrations are elevated in patients with reactive airway disease. In: Biology of Ntitric Oxide: Clinical and Physiological Implications. London: Portland Press, 1994:497–500.
43. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide

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in exhaled air of asthmatic patients. Am J Respir Crit Care Med 1996; 153:454–457.
44. Frank TL, Adisesh A, Pickering AC, Morrison JF, Wright T, Francis H, Fletcher A, Frank PI, Hannaford P. Relationship between exhaled nitric oxide and childhood asthma. Am J Respir Crit Care Med 1998; 158:1032–1036.
45. Simpson A, Custovic A, Pipis S, Adisesh A, Faragher B, Woodcock A. Exhaled nitric oxide, sensitization, and exposure to allergens in patients with asthma who are not taking inhaled steroids. Am J Respir Crit Care Med 1999; 160:45–49.
46. Silkoff PE, Romero F, Milgrom H, Tran ZV, Townley R, Gupta N, Rohane P, Fick R. Exhaled nitric oxide (eNO) and anti-inflammatory effects of a recombinant humanized monoclonal antibody to IgE (rhuMAb-E25) in pediatric asthma. Chest 2000; 118(4):101S.
47. Togias A, Corren J, Shapiro G, Reimann JD, von Schlegell, Wighton TG, Adelman DC. Anti-IgE treatment reduces skin test (ST) reactivity. J Allergy Clin Immunol 1998; 101:S171.
48. Durahm SR, Gould HJ, Hamid QA. Local IgE production in nasal allergy. Intl Arch Allergy Immunol 1997; 113:128–130
49. Corren J, D Diaz-Sanchez, J Reimann, A Saxon, D Adelman. Effects of anti-IgE antibody therapy on nasal reactivity to allergen and IgE synthesis in-vitro. J Allergy Clin Immunol 1998; 101:S105.
50. Nahm DH, Park HS. Analysis of induced sputum for studying allergen-specific IgE antibodies in airway secretion from asthmatic patients. Clin Exp Allergy 1998; 28(6): 686–693.
51. Chvatchko Y, Kosco-Vibois MH, Herren S, Lefort J, Bonnefoy JY. Germinal center formation and local immunoglobulin E (IgE) production in the lung after an airway antigenic challenge. J Exp Med 1996; 184:2353–2360.
52. Campbell AM, Vignola AM, Chanez P, Godard P, Bousquet P. Low affinity receptor for IgE on human bronchial epithelial cells in asthma. Immunology 1994; 82:506–508.
53. Letellier M, Delespesse G, Sarfati M, Wu CY, Fournier S. The low-affinity receptor for IgE. Immunol Rev 1992; 125:77–97.
54. Metzger H. The receptor with high affinity for IgE. Immunol Rev 1992; 125:37.
55. Ishizaka K, Conrad DH, Schulman ES, Sterk AR, Ishizaka K. Biochemical analysis of initial triggering events of IgE-mediated histamine release from human lung cells. J Immunol 1983; 130:2357–2362.

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14

Pulmonary Delivery of Anti-IgE

Rationale for Topical Delivery to the Airway

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I. Introduction

Topical administration of inhaled therapeutics is the current standard for the therapeutic management of asthma. Pressurized metered dose inhalers (pMDIs) or dry powder inhalers (DPIs) containing either steroids to combat inflammation or bronchodilators for rescue airway dilation are routinely used by the majority of asthma patients. The rationale for a topical course of treatment is obvious: to maximize the dose of the therapeutic agent to the target tissue site, the asthmatic lung, and to minimize systemic side effects. Other routes of administration, i.e., oral administration, are used for agents with low systemic toxicity (e.g., leukotriene antagonists) or where topical therapy does not adequately achieve desired therapeutic outcome (e.g., steroids). For proteins, oral therapy is not possible due to degradation of the therapeutic agent in the gastrointestinal tract.

During the development of anti-IgE (E25) as a therapeutic for the treatment of allergic asthma, we wondered if increasing the concentration of E25 by topical delivery to the lung surface would have a therapeutic benefit. However, unlike steroids and bronchodilators, the target for E25 (IgE) is known to be present not only in the lung tissue but also throughout the systemic circulation. This chapter

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will describe the rationale for delivering E25 locally to the lung and the challenges in understanding the role of lung versus systemic IgE in allergic asthma.

A. Rationale for Topical E25 Therapy

For decades it has been known that patients with allergic asthma have elevated IgE levels in both their serum as well as in the epithelial lining fluid (ELF) of their lungs (1–3). Earlier chapters in this text review the evidence for the role of local and serum IgE in asthma, so this evidence will not be reviewed here. Since IgE is present in both the ELF and serum of patients with asthma, it seemed reasonable that the target for E25 was located both systemically and topically. Yet the relative role of local, compared to systemic, IgE in the asthmatic response was unknown.

The allergic response is precipitated when IgE bound to the high-affinity receptor on mast cells and basophils is cross-linked by a specific allergen, resulting in the release of mediators such as histamine and leukotrienes. The response culminates in bronchoconstriction, inflammation, and increased vascular permeability. The cells involved in the allergic response are recruited and then localized in lung tissue of asthmatics. Laitinen and colleagues (4) sampled the airway epithelium by bronchcoscopy from normal and asthmatic patients. Asthmatic airways had a 6- to 75-fold increase in the number of lymphocytes, mast cells, and eosinophils per mm^2 airway compared to normals. Additionally, IgE-bearing basophils are present in the ELF of the human airways during the late-phase response to antigen challenge (5). Furthermore, the allergens that are responsible for the allergic response are presented to the airway by inhalation. These data suggest that all of the components necessary to elicit the asthmatic response are localized in the airway and that airway IgE may be the most important pool.

It is also known that cells in the lung produce antibodies, particularly IgE antibodies in asthma (6,7). Yamaguchi and colleagues (7) found that IgE was present in higher concentrations in the bronchoalveolar lavage (BAL) fluid from asthmatics compared to control patients. These data support the suggestion that local IgE production may be the most relevant pool responsible for the asthmatic response. Crimi and colleagues (8) measured specific IgE levels in bronchial lavages and serum of human subjects. Their data suggested that airway IgE levels were not always correlated with serum IgE levels. Deushl (9) also suggested that serum IgE levels do not always reflect the immunological status of an organ.

One final piece of evidence to support topical delivery of E25 is from Bice and Muggenburg (10). These scientists gave keyhole limpet hemocyanin (KLH) antigen to the left cardiac lobe (LCL) and sheep red blood cell (SRBC) antigen to the right cardiac lobe (RCL) of dogs. Three weeks later the LCL and RCL were again challenged with the same antigen. After the first antigen challenge,

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B lymphocytes recovered by BAL produced equivalent quantities of antibodies to either antigen regardless of the lung lobe measured. These data suggest that after a primary antigen exposure, antibody-producing cells are recruited into the lung from the blood. Following the second antigen challenge, B lymphocytes lavaged from the LCL produced an order of magnitude greater quantity of antibodies of KLH than SRBC. Conversely, B lymphocytes lavaged from the RCL produced an order of magnitude greater quantity of SRBC antibodies than KLH antibodies. These data suggest that memory B lymphocytes are localized in the lung interstitium at the site of a prior antigen challenge. Local antibody production is primarily from these local B memory cells and to a lesser extent from B lymphocytes recruited from the blood.

If we assume that the lung may be the primary source of IgE in BAL and interstitium and that the cells that respond to IgE are localized in the lung then the lung would be the primary site to neutralize IgE. Topical delivery of E25 to the lung was the best strategy to bind and thereby neutralize the IgE present in the ELF of the lung. To sufficiently neutralize IgE in the interstitial space with topical E25, the antibody would likely need to be transported across epithelium and bind to IgE present in the interstitium. We performed both in vitro experiments using monolayers of epithelial cells and in vivo experiments using both rodents and nonhuman primates to evaluate epithelial cell permeability to E25.

II. Permeability of Lung Epithelium to E25

To neutralize the interstitial pool of IgE, the pulmonary epithelium would need to be permeable to E25. Since E25 is a humanized antibody of the IgG1 subclass, it should translocate across epithelial barriers similar to other IgG antibodies. Although the mechanisms of immunoglobulin (Ig) transepithelial transport across lung epithelium are not clear, it is known that Igs are located in the ELF of the lung and Ig levels have been measured and characterized in BAL samples from normal and diseased human lungs (3,11,12). The two primary immunoglobulins in the ELF from normal humans are IgG1 and IgA. These represent 10–15 and 5%, respectively, of the total protein recovered from the BAL of a normal human lung (11). IgE is not normally detected in the ELF of the human lung; however, disease changes the immunoglobulin composition, often shifting the profile toward increased IgE in atopic asthmatics (3). Immunoglobulins found in the ELF are most likely produced in lung tissue. Hance and colleagues (6) showed that minimal immunoglobulins were produced de novo by B lymphocytes recovered from BAL of human subjects, but cells recovered from lung tissue were capable of producing immunoglobulins. These authors suggest that lung tissue is the source for the immunoglobulins in the ELF. However, in dogs B lymphocytes recovered by BAL were capable of producing antibodies in vitro (10). If indeed

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most Igs are produced in the lung interstitium in humans, then these immunoglobulins must transport across the epithelium to reach the luminal surface. The epithelium presents a formidable barrier for large macromolecular transport. Full-length immunoglobulins are at a minimum 150 kDa and are not predicted, based on their size, to traverse through tight junctions. Patton and colleagues (13) suggest that the highest transepithelial transport occurs for macromolecules of approximately 18–33 kDa.

Although there are two known immunoglobulin transporters, the poly Ig receptor for secretory IgA (14) and the FcRn receptor for IgG (15,16), they most likely do not play a major role in E25 transport across lung epithelium as evidenced by the data described below.

A. In Vitro Studies: Airway and Alveolar Cell Culture Models

We used in vitro models of lung epithelial cell lines or primary cells to determine E25 permeability. Two different cell types were used to mimic the airway and alveolar epithelium of the lung to study transport. Calu-3, a human cell line derived from an airway carcinoma, when grown at an air/liquid interface, differentiate to form a secretory airway epithelium (17). Rat primary epithelial cells isolated as described by Cheek et al. (18) form a tight barrier similar in structure and function to the alveolar surface. Both cell types when grown to confluence form tight junctions and differentiate and polarize so that the apical or air surface has different characteristics than the basolateral or ''blood'' side. The typical transepithelial resistance observed was 350 or >1000 ohms-cm^2 for Calu-3 cells or primary rat alveolar cells, respectively. Once an acceptable resistance was achieved, E25 (2 mg/mL) was placed in either the apical or the basolateral chamber. Cell monolayers were incubated at 37°C for up to 3 hours and ELISA measured the amount of E25 that translocated the epithelial layer and appeared in the receiver well. The apparent permeability (Papp) of the epithelium for E25 was calculated as:

$$\text{Papp(cm/sec)} = (dM/dt) * [1/A * C]$$

where M is total mass of transported protein (mg), t is time (s), A is surface area (1.13 cm^2), and C is concentration in the donor compartment (mg/cm^3).

E25 had similar permeability in both cell types and 10-fold higher permeability in the basolateral to apical direction than in the apical to basolateral direction (see Table 1). In either direction, permeability across airway or alveolar epithelium to the antibody (E25) was low. Similar transport rates were obtained by Kim and Crandall (19) for an IgG antibody in rat alveolar epithelial cells, although directional preferences for transport were the opposite of those observed in Table 1.

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Table 1 Permeability of Lung Epithelial Cells to E25

	Papp, apical to basolateral	Papp, basolateral to apical
Calu-3	5.4 ± 4.5	51 ± 14
Rat alveolar	4.9 ± 2.5	67 ± 16

Papp = Apparent permeability mean ± SD ($\times 10^{-8}$ cm s^{-1}).
Cells were grown to confluence on Transwell filters. Calu-3 cells are an airway epithelial cell line; rat alveolar cells are a primary culture of alveolar epithelium. Experiments were run in triplicate.

B. In Vivo Studies: Rodents and Nonhuman Primates

In vivo experiments in rodents and monkeys also showed that E25 transport across the epithelial surface was low. Initial experiments were performed in rodents. Rats were given E25 by either aerosol administration (35 μg deposited in the lung) or intravenous injection (1 mg/kg; 250 μg). For the aerosol exposure, rats were placed in a nose-only inhalation chamber (Jaeger) and exposed to E25 for 30 minutes. E25 aerosols were generated using a Pari IS-2 nebulizer at an operating pressure of 22 psi. Lung dose was estimated from inhalation studies using radiolabeled aerosols of comparable particle size and normalizing for the amount of E25 or radiolabeled surrogate that exited the nebulizer during a 30-minute aerosol exposure. One hour after E25 administration, animals were euthanized, their lungs were cleared of blood by perfusion, and the lung linings washed extensively by BAL to remove ELF and blood. Following lavage and perfusion, the lung tissue was minced. E25 levels in BAL fluid, serum, and lung tissue homogenate were measured by ELISA. Figure 1 shows that 0.01% of the E25 administered by the IV route was detected in the BAL of the rat. After aerosol administration there was substantial lung tissue levels of E25, but none was detected in serum.

In addition to rat experiments, experiments in the mouse were also conducted to determine the kinetics of transport into the lung following IV administration. Mice were given a single IV injection of 1 mg/kg (20 μg) of E25, and then BAL was performed at various times postadministration. Separate mice were used for different time points. As in the rat studies, BAL levels of E25 in the mice were approximately 10,000 times lower than serum levels following bolus IV administration (Fig. 2).

Repeated daily E25 aerosol administration was studied in both the rat (116 μg/kg deposited/day) and the cynomolgus monkey (67 μg/kg deposited). Exposures were by nose-only inhalation for the rat and head-only inhalation for the monkey studies. E25 aerosols were generated in both studies using a Pari-IS2 nebulizer. It required multiple days (approximately 5) of aerosol exposure in both species before E25 was detected in the serum (Figs. 3 and 4). These animal

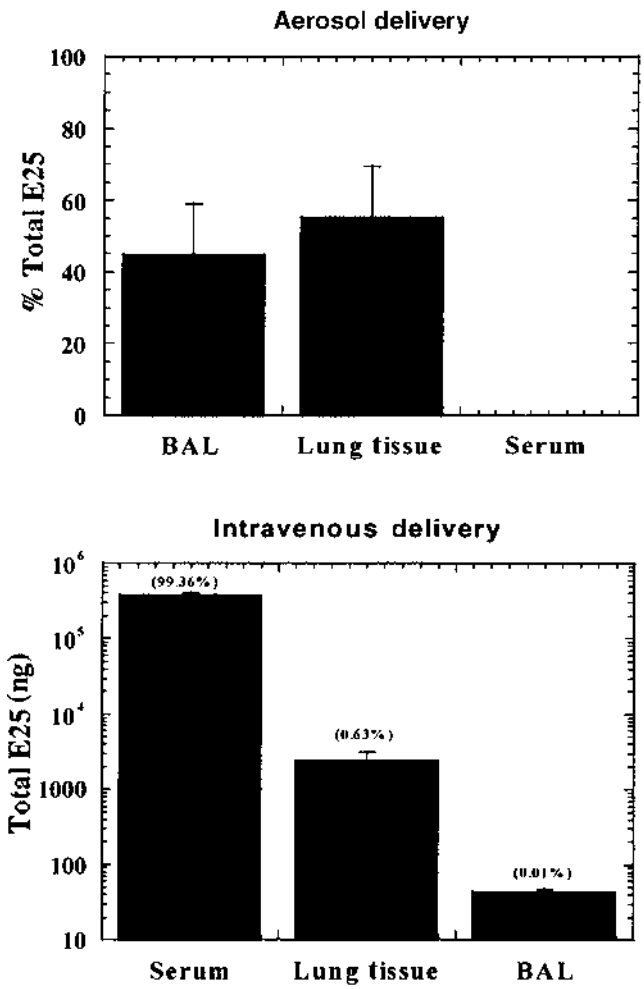

Figure 1 Distribution of E25 in bronchoalveolar lavage (BAL) fluid, lung tissue, and serum 1 hour following a single aerosol (top; 35 μg deposited) or IV (bottom; 250 μg) administration in the rat. (From Ref. 20.)

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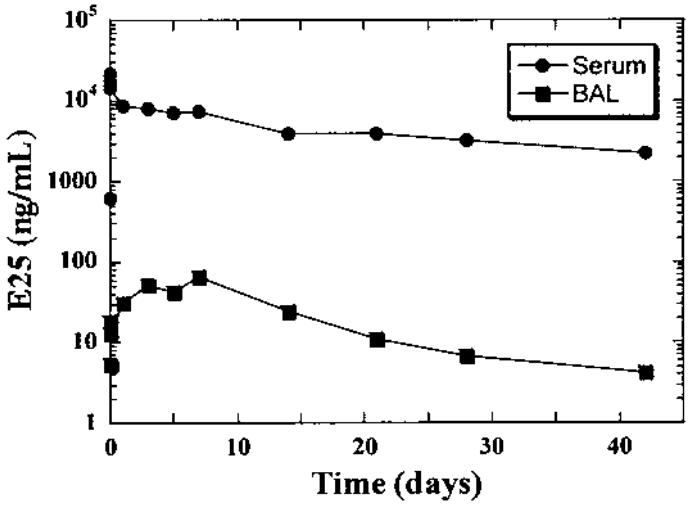

Figure 2 E25 levels in the serum and bronchoalveolar lavage (BAL) fluid of the mouse following a single IV administration (1 mg/kg; 20 μg). (From Ref. 20.)

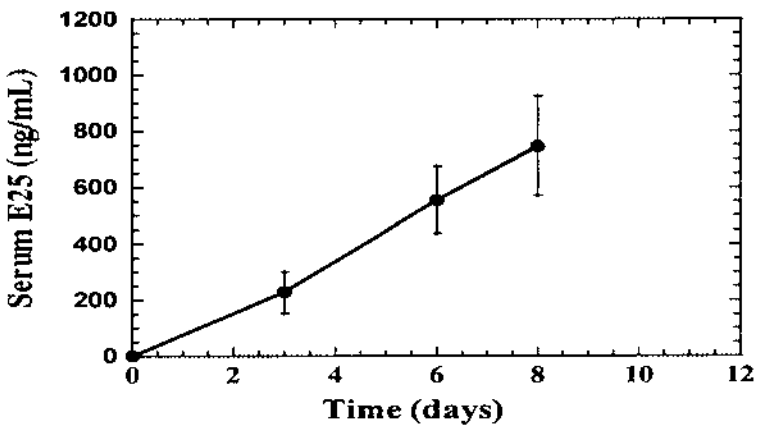

Figure 3 E25 levels in the serum following a daily aerosol administration in the rat. Rats were exposed to an E25 aerosol generated by a Pari-IS-2 nebulizer for 30 min/day for 7 days in a nose-only inhalation chamber. 116 μg/kg of E25 deposited in the lungs of the rats per day. (From Ref. 20.)

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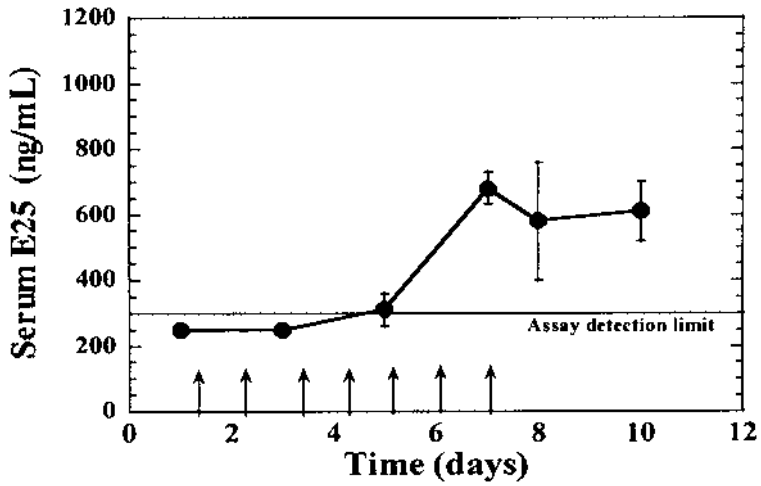

Figure 4 E25 levels in the serum of the monkey following aerosol administration using a Pari-IS-2 nebulizer daily for 7 days. Approximately 67 μg/kg of E25 deposited in the lungs per day. (From Ref. 20.)

studies confirmed that E25, a 150 kDa IgG antibody, does transport across the lung epithelium. Transport was low and similar in all species, suggesting a non-specific inefficient transport mechanism.

III. Dose Estimation and Safety of Aerosolized E25

In vitro and in vivo studies suggested that aerosolizing E25 would deliver the therapeutic primarily to the ELF with low and limited delivery to the serum. The quantity delivered to lung interstitium could not be determined. To evaluate the potential efficacy of topical delivery to the lung surface and to estimate dose, a suitable animal model would be advantageous. However, no suitable animal model was available. Rodent models of asthma could not be used to evaluate efficacy since E25 does not bind to rodent IgE. Also, although E25 does bind to monkey IgE, at the time of these studies there was no well-characterized monkey model of asthma. We decided that the fastest way to evaluate the efficacy of aerosolized E25 was to use a human bronchoprovocation model of asthma since systemic delivery of E25 was found to be efficacious in this model (21,22).

To evaluate aerosolized E25 in humans, an estimated dose and the safety of inhaled E25 needed to be determined. Cynomolgus monkeys were used for these estimates.

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Pharmacokinetics of Aerosolized E25 in ELF

The dose of inhaled E25 was estimated from phamacokinetic studies in cynomolgus monkeys. Monkeys were exposed to a single dose of aerosolized E25 for 20 minutes by head-only inhalation using a Pari-IS2 nebulizer. Total volume inhaled during the aerosol exposure was measured and the concentration of E25 in the inhaled air was determined by filter sampling. Aerosol droplet size was also measured by cascade impaction. The estimated deposited dose in the lungs from these determinations was 117 μg. To evaluate the kinetics of E25 deposited on the lung surface, samples of the ELF were taken by BAL and the total E25 concentration measured by ELISA. Samples of BAL were taken at 0, 1, 2, 4, 8, 12, and 24 hours postinhalation. Only two BAL samples were taken from a single animal. Two animals were used for each time point. Urea concentrations in BAL fluid were used for dilution corrections to estimate ELF (23).

Cynomolgus monkeys have elevated IgE levels in both their serum and BAL fluid. Since the levels are comparable to those found in human subjects with allergic asthma, we measured the ratio of E25 to IgE attained in the BAL fluid. A single lung dose of 117 μg (Fig. 5) was sufficient to maintain a greater than 10:1 ratio of E25 to IgE in the ELF of these animals for 24 hours. Previous studies showed that a 10:1 ratio of E25 to IgE was necessary for efficacy. This data suggested that once-daily dosing of E25 would be sufficient to neutralize IgE in lung lining fluid.

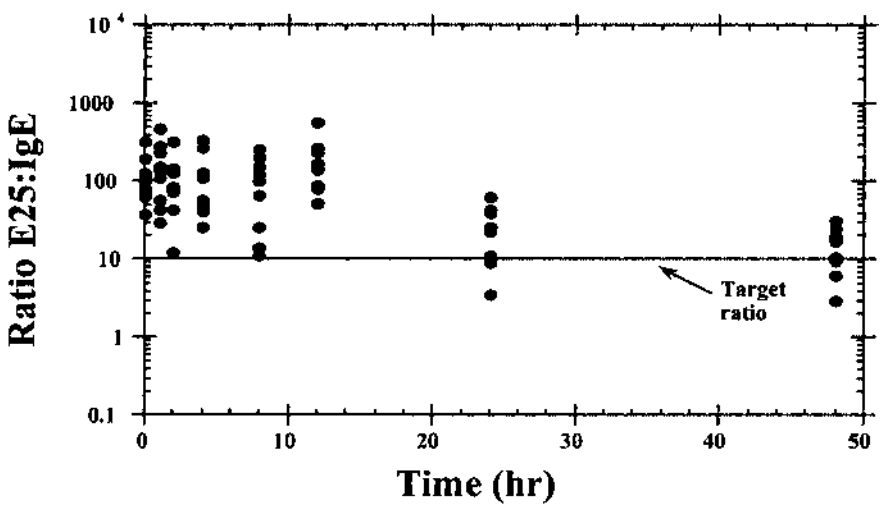

Figure 5 Ratio of E25 to IgE concentrations in BAL from monkeys following a single aerosol inhalation of E25. Estimated dose deposited from air sampling was 117 μg.

B. Safety of Aerosolized E25

Cynomolgus monkeys were used to evaluate the safety of inhaled E25. Two studies, 7- and 60-day exposure, were performed to evaluate the safety of this novel therapeutic prior to administration to humans. General safety and tissue responses along with potential antigenicity to inhaled E25 were evaluated. Development of antibodies directed against E25 would be expected in these safety studies due to the differing homology between the humanized monoclonal antibody (E25) and monkey IgG (24).

A pilot 7-day toxicity study with aerosolized E25 was conducted in cynomolgus monkeys to evaluate lung pathology following daily head-only inhalation exposure to E25 (400 μg/day) or E25 vehicle. Total E25 concentrations in BAL were approximately 250-fold greater than IgE concentrations, and serum levels were comparable to those in Figure 4. There were no test material–related effects on clinical observations, body weights, or body temperature throughout the study. There was a minor change in lung cytology as measured by BAL with a slight increase in neutrophils in the treatment groups compared with vehicle control animals. There were no major differences in gross or microscopic lesions found in the larynx, trachea, or lungs of the animals exposed to E25 compared to control animals exposed to E25 vehicle. The minor inflammation seen in the lungs was most likely due to the BAL procedure. Based on the results of this study, aerosolized E25 at deposited doses up to 400 μg had no effect on lung pathology or any other parameters measured.

Subsequently, a 60-day repeated dose inhalation toxicity study of aerosolized E25 was conducted in cynomolgus monkeys. The study consisted of four dose groups (4/sex/group), plus two monkeys/sex in the control and high-dose groups, which were allowed to recover for 30 days following the 60-day exposure. Cynomolgus monkeys were exposed to E25 aerosols generated by a Pari LC Jet Plus nebulizer. The initial solution concentrations were 0.5, 5, or 20 mg/mL E25 to achieve a targeted bronchopulmonary deposition of 10, 100, or 400 μg. Representative filter samples of air concentration in the breathing zone of the monkey were obtained to assure the delivery of the desired dose to the lungs. The generation and exposure system performed as designed, with animals receiving within 10% of the targeted doses, and the mass median aerodynamic diameter of the aerosol in all groups was between 1.8 and 2.2 μm.

Exposure to aerosol E25 for 60 consecutive days was well tolerated, and there were no treatment-related effects on clinical observations, physical examinations, food consumption, body weights, ophthalmic examinations, clinical pathology, clinical chemistry, urinalysis, BAL cytology, heart rate, blood pressure, or ECG.

The main focus of this safety study was to assess the safety of local delivery to the lung. There was an antibody response to E25 (discussed below); thus most

organs and tissues were carefully checked for the presence of lymphoid cell infiltrations and aggregates. Other studies have shown an increase in mononuclear cell infiltrates in the lung and airway tissues, and large localized perivascular lymphoid follicles appearing in lung tissue after multiple aerosol antigen challenge (25,26). Therefore, the intent was to identify changes in lymphoid cell populations, if any.

Despite such coding of lymphoid cell infiltrations in various organs, no distinctive or consistent response to E25 was found. Pharyngeal, nasal, and tonsilar lymphoid tissues all had relatively normal lymphoid cell populations, regardless of whether the animal was exposed to E25 or vehicle. Half of the monkeys, including controls, had evidence of germinal center activity within one or more peribronchial lymphoid aggregates, which were coded as minimal. However, two or more sites of activity were identified for one control, one low-dose, and four high-dose animals. It is unclear whether this tendency to have slightly more germinal center activity in the highest exposure level reflects a true response to E25. Since the nature of the response was similar in control as well as exposed monkeys, the significance of this response is likely to be negligible.

A more unique observation was a minimal eosinophil cell infiltration associated with the bronchial mucosa in one mid-dose animal and three high-dose animals. No such infiltrates were found in the vehicle control monkeys. No mucosal tissue alterations were observed coincident with the eosinophil infiltrates. Three different monkeys from the low- and mid-dose groups had slightly higher eosinophil percentages in the BAL samples taken just prior to necropsy. This minimal effect in a few monkeys may reflect a mild response to the inhalation of a foreign particulate or an immunological response to E25. Most animals also had microscopic evidence of peracute trauma and inflammation due to the BAL procedure conducted just prior to necropsy. The erosive lesions of the larynx, trachea, and/or bronchi occurred in controls and exposed alike that had undergone the BAL procedure.

Antibodies to E25

Serum samples were evaluated for the presence of antibodies to E25 by two ELISA assays, one for anti-Fab antibody and another for anti-Fc detection. Cynomolgus monkey serum samples were equally diluted. Assay sensitivity was $\log_{10}$ 100 or 2.0 titer units. Assay interference due to E25-IgE complexes and high E25 concentrations prevented evaluation of all BAL samples and serum samples with IgE concentrations greater than 15 μg/mL.

By day 61, most treated animals that could be assayed developed serum antibody titers (30/31 had anti-Fab titers and 11/31 had anti-Fc titers) to aerosolized E25. Anti-Fab titers ranged from 2.1 to 5.7; anti-Fc ranged from 2.2 to 3.3. Some animals in the control group were also positive for serum antibodies to

E25 (4/11 anti-Fab and anti-Fc). Titers in these control animals ranged from 2.9 to 4.5 for anti-Fab and from 2.2 to 3.2 for anti-Fc. The anti-Fab antibodies appeared to be specific for E25 because addition of E25 to the serum samples significantly reduced the titers. Two other control animals tested false positive for serum antibodies (anti-Fab) prior to study start. Antibody titers have been seen in previous studies with subcutaneous and intravenous administration of E25. Since high levels of IgE (>15 μg/mL) in addition to E25 in the serum can cause a false positive in the antibody assay, many of the samples from previous subcutaneous/intravenous studies could not be assayed and total antibody incidence could not be established.

Others have shown that animals exposed to aerosol antigens generate a greater antibody response than animals exposed to the same intravenous dose (27). However, although a high incidence and magnitude of antibody titers against E25 were seen in this study, there were no apparent pathologies in the lung or kidneys associated with formation of immune complexes (anti-E25-E25-IgE complexes). In addition, there was no evidence of hypersensitivity responses in any of these animals, and no real evidence of increased cellular influx or increased activation of lymphoid cells in the lung as described previously.

IV. Human Clinical Studies

Studies in animal models predict that topical therapy with E25 would be sufficient to neutralize the pool of IgE in the ELF. Due to the low permeability of the epithelium for E25, topical delivery would not be sufficient to neutralize the serum IgE pool. Human clinical trials were designed to test whether neutralizing the lung pool of IgE by aerosol delivery of E25 would be efficacious in a bronchoprovacation model of human asthma (28). A randomized, double-blind study in 33 subjects with mild allergic asthma (age range 20–46 years; FEV_1 > 70% predicted) was performed. Three groups were treated once daily with either placebo ($n = 11$), low dose (1 mg in the nebulizer; $n = 12$) or high dose (10 mg; $n = 10$) of aerosolized E25 daily by jet nebulization. Nebulization of E25 was accomplished using a PARI IS-2 jet nebulizer powered by a PARI master compressor. The estimated lung dose of aerosolized E25 was 0.15 or 1.5 mg/day, respectively, for the low- and high-dose groups. Airway responses to aerosolized allergen were determined at baseline and after either 2 or 8 weeks of treatment and at 4 weeks following the completion of treatment (washout). Nonspecific airway responses to methacholine were also measured at these time points.

Aerosolized E25 was safe and well tolerated in the subjects; no significant changes in AM peak flow, in FEV_1, or in methacholine responses were observed

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in either the low- or high-dose groups. Yet, despite daily inhalation of E25, the early (EAR) or late phase response (LAR) to an inhaled allergen in these patients was not affected (28). Treatment with the low dose of anti-IgE did have a significant within group attenuation in the EAR, but this change was not significantly greater than placebo. The high-dose group did not show a significant within-group effect. Both doses of E25 were no more effective than placebo in attenuating the early or late phase response to allergen.

Bronchoscopy was performed and bronchoalveolar lavage samples of epithelial lining fluid (ELF) taken in 10 of the subjects. Lung E25 levels in BAL fluid were high enough (>10-fold) to neutralize the topical IgE pool, but as anticipated serum E25 levels (Fig. 6) were not sufficient to neutralize serum IgE levels (mean 0.63 μg/mL). After daily aerosol delivery using a Pari-IS-2 nebulizer containing 10 mg of E25 with a Pari Master compressor, serum levels were measurable by day 14 (the first time point in the study) and continued to rise throughout the course of therapy in most patients (Fig. 6) (28). One patient developed a neutralizing serum titer to E25 in the aerosol study. Neutralizing antibodies have not been detected in any of the patients treated systemically (either IV or SC administration) with E25. These data suggest that the rhuMAb E25 antibody may have increased immunogenicity in asthmatic subjects by the aerosol route of administration. More data would need to be collected to confirm these findings.

Because intravenous therapy was successful in EAR and LAR improvement in a similar study design (21,22), these results suggest that it is important

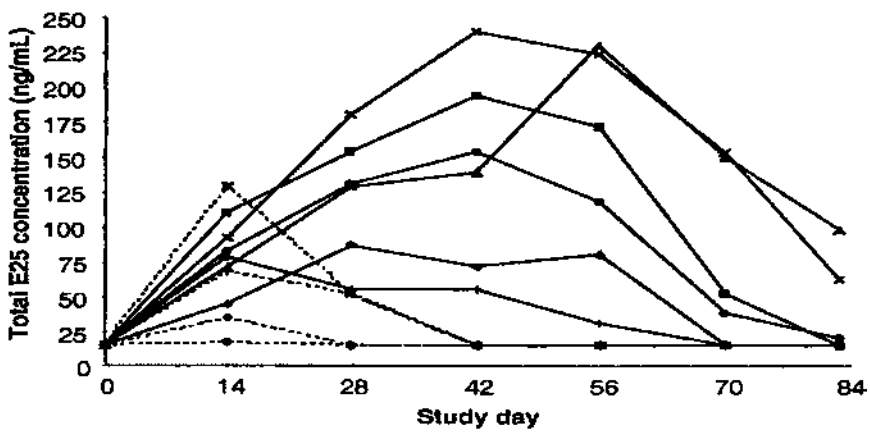

Figure 6 E25 levels in the serum of allergic asthmatics after daily aerosol administration using a Pari-IS-2 nebulizer containing 10 mg of E25 and a Pari Master compressor (1.5 mg E25 deposited in the lung/day). Each line represents a single patient. (From Ref. 24.)

to neutralize the serum IgE pool in order to ameleorate the symptoms of asthma in allergic patients. Another possible interpretation is that aerosolized E25 is more immunogenic than systemic therapy (28).

V. Summary and Conclusions

Unlike other therapeutic regimens for the treatment of asthma, E25 therapy is not effective by topical administration. The data in rodents and nonhuman primates support that aerosolized therapy will neutralize IgE in ELF, but because the lung epithelium has low permeability to antibodies, topical therapy using feasible inhaled doses of E25 cannot neutralize IgE in the serum. These data suggest that both the serum and lung IgE pools are important contributors to the allergic asthmatic response.

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References

1. Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. N Engl J Med 1991; 325:1067–1071.
2. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TA. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989; 83:875–882.
3. Fick RB, Richerson HB, Zavala DC, Hunninghake GW. Bronchoalveolar lavage in allergic asthmatics. Am Rev Respir Dis 1987; 135:1204–1209.
4. Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. Am Rev Respir Dis 1993; 147:697–704.
5. Guo C-B, Liu MC, Galli SJ, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Identification of IgE-bearing cells in the late-phase response to antigen in the lung as basophils. Am J Respir Cell Mol Biol 1994; 10:384–390.
6. Hance AJ, Saltini C, Crystal RG. Does de novo immunoglobulin synthesis occur on the epithelial surface of the human lower respiratory tract? Am Rev Respir Dis 1988; 137:17–24.
7. Yamaguchi E, Sito S, Okazaki N, Abe S, Kawakami Y. Plasma cells in the bronchoalveolar lavage fluid of a patient with eosinophilic pneumonia. Morphologic proof of the local production of antibodies. Chest 1988; 93:110–113.
8. Crimi E, Scordamaglia A, Crimi P, Zupo S, Barocci S. Total and specific IgE in

serum, bronchial lavage and bronchoalveolar lavage of asthmatic patients. Allergy 1983; 38:553–559.
9. Deuschl H. Immunoglobulins in nasal secretion with special reference to IgE. Seasonal studies of Timothy-specific IgE-antibody in patients with allergic rhinitis. Int Arch All Appl Immunol 1976; 52:376–383.
10. Bice DE, Muggenburg BA. Pulmonary immune memory: localized production of antibody in the lung after antigen challenge. Immunology 1996; 88:191–197.
11. Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. J Lab Clin Med 1974; 84:559–573.
12. Merrill WW, Naegel GP, Olchowski JJ, Reynolds HY. Immunoglobulin G subclass proteins in serum and lavage fluid of normal subjects. Am Rev Respir Dis 1985; 131:584–587.
13. Patton JS, Nagarajan S, Clark A Pulmonary absorption and metabolism of peptides and proteins. In: Dalby RN, Byron PR, Farr SJ, eds. Respiratory Drug Delivery VI. Buffalo Grove, IL: Interpharm Press, Inc., 1998:17–24.
14. Mostov KE. Transepithelial transport of immunoglobulins. Annu Rev Immunol 1994; 12:63–84.
15. Raghavan M, Bjorkman PJ. Fc receptors and their interactions with immunoglobulins. Annu Rev Cell Dev Biol 1996; 12:181–220.
16. Simister NE, Jacobowitz Israel E, Ahouse JC, Story CM. New functions of the MHC class I-related Fc receptor, FcRn. Biochem Soc Trans 1997; 25:481–486.
17. Shen BQ, Finkbeiner WE, Wine JJ, Mrsny RJ, Widdicombe JH. Calu-3: a human airway epithelial cell line that shows cAMP-dependent Cl-secretion. Am J Physiol 1994; 266:L493–501.
18. Cheek JM, Evans MJ, Crandall ED. Type I cell-like morphology in tight alveolar epithelial monolayers. Exp Cell Res 1989; 184:375–387.
19. Kim KJ, Crandall ED. Role of endocytic pathways in alveolar epithelial transport of macromolecules. In: Dalby RN, Byron PR, Farr SJ, eds. Respiratory Drug Delivery V. Buffalo Grove, IL: Interpharm Press, Inc., 1996:153–159.
20. Sweeney TD, Marian M, Achilles K, Bussiere J, Ruppel J, Shoenhoff M, Mrsny R. Biopharmaceutics of immunoglobulin transport across lung epithelium. In: Dalby RN, Byron PR, Farr SJ, Peart J, eds. Respiratory Drug Delivery VII. Raleigh: Serentec Press, Inc., 2000:59–66.
21. Fahy JV, Fleming E, Wong HH, Liu JT, Su JQ, Reimann J, Fick RB, Boushey HA. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med 1997; 155: 1828–1834.
22. Boulet L-P, Chapman KR, Côté J, Kalra S, Bhagat R, Swystun VA, Laviolette M, Cleland LD, Deschesnes F, Su JQ, DeVault A, Fick RB, Cockcroft DW. Inhibitory effect of an anti-IgE antibody E25 on allergen-induced early asthmatic response. Am J Respir Crit Care Med 1997; 155:1835–1840.
23. Rennard SI, Basset G, Lecossier D, O'Donnell, KM, Pinkston P, Martin PG, Crystal RG. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. J Appl Physiol 1986; 60:532–538.
24. Green JD. Pharmaco-toxicological expert report Pulmozyme rhDNase. Human Exp Tox 1994; 13(suppl 1):S1–42.

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25. Thepen T, McMenamin C, Girn B, Kraal G, Holt PG. Regulation of IgE production in pre-sensitized animals: in vivo elimination of alveolar macrophages preferentially increases IgE responses to inhaled allergen. Clin Exp Allergy 1992; 22:1107–1114.
26. Mason MJ, Gillett NA, Bice DE. Comparison of systemic and local immune responses after multiple pulmonary antigen exposures. Regional Immunol 1989; 2: 149–157.
27. Yoshizawa Y, Hostetter MW, Nakazawa T, Ripani LM, Dawson CA, Moore VL. Enhancement of systemic immune response by immunization into chronically inflamed lungs. J Lab Clin Med 1982; 100:61–69.
28. Fahy JV, Cockcroft DW, Boulet LP, Wong HH, Deschesnes F, Davis EE, Ruppel J, Su JW, Adelman DC. Effect of aerosolized anti-IgE (E25) on airway responses to inhaled allergen in asthmatic subjects. Am J Respir Crit Care Med 1999; 160: 1023–1027.

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15

Treatment of Seasonal Allergic Rhinitis

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I. Introduction

Allergic rhinitis, an inflammatory disorder, results from the interaction between aeroallergens and allergen-specific immunoglobulin E (IgE) bound to mast cells and basophils in the mucosal tissues of the upper airways of sensitized individuals. Symptoms include sneezing, rhinorrhea, nasal congestion, and pruritus of the nose, eyes, ears, and throat. These symptoms may occur with seasonal periodicity when they are triggered by tree, grass, or weed pollens or outdoor mold spores, but they may also be more continuous when caused by perennial allergens such as house dust mites, animal danders, occupational allergens, cockroach, and indoor mold spores. The pathogenesis of seasonal allergic rhinitis and perennial allergic rhinitis appears to be similar, and therapeutic principles for one can be applied to the other.

In the Western Hemisphere, allergic rhinitis affects 15–25% of the population (1,2), and it appears that the prevalence of allergic sensitization has been increasing over the last few decades (3,4). Most patients develop allergic rhinitis prior to adulthood, but a significant proportion develop it as adults. Pollution, viral infections, gender, genetic predisposition, geography, and socioeconomic status contribute to the onset and severity of symptoms.

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A mere nuisance to some, allergic rhinitis can be temporarily debilitating or incapacitating to others and may contribute to the development of otitis media (5), sinusitis (6), and asthma exacerbations (3). Quality of life is adversely affected in rhinitic patients (7,8). Furthermore, the direct expenditures for the treatment of allergic rhinoconjunctivitis can be measured in the billions of dollars (3,9–11).

Today a vast number of medications are available or under study for the treatment of allergic rhinitis. This chapter will focus on the pathophysiology of allergic rhinitis and the pharmacological rationale behind current and emerging therapies.

II. Pathophysiology

A. Overview

Allergic rhinitis is a type I hypersensitivity disease, and its pathogenesis occurs in three stages: (1) sensitization, (2) reexposure or provocation resulting in the acute (early phase) allergic reaction, and (3) the late phase reaction and chronic inflammation. The pathogenesis of allergic rhinitis involves many different cell types, inflammatory mediators, cytokines, chemokines, and adhesion molecules.

Sensitization begins with the generation of allergen-specific IgE. Airborne allergens settle on the nasal mucosa, where antigenic fragments are processed by Langerhans cells (12) and are presented as oligopeptides to T-helper (Th) cells. Allergen-specific B cells switch to IgE antibody production under the influence of interleukin-4 (IL-4) and IL-13 produced by Th2 and other cells (13). The sensitization process is completed when allergen-specific IgE antibodies bind via high-affinity Fcε receptors (FcεRI) to the surface of mast cells and basophils residing in the mucosal tissues of the upper respiratory tract.

Sensitization is a silent event, but reexposure or provocation is anything but silent. Upon reexposure, the allergen binds to cell surface–bound IgE, resulting in cross-linking of the IgE:FcεRI complexes, which activates mast cells and basophils. Activation results in (1) degranulation with release of preformed mediators including histamine, various proteases (e.g., tryptase) and cytokines, and (2) synthesis and release of newly generated lipid-derived mediators and cytokines. The released mediators affect nerve endings, blood vessels, and submucosal glands. An immediate (early phase) response, primarily mediated by histamine and neural reflexes, is then followed by a more prolonged inflammatory (late phase) response involving many inflammatory cells and their mediators (Fig. 1).

B. Early- and Late-Phase Allergic Responses

The early phase response in allergic rhinitis is characterized by sneezing, nasal itching, nasal congestion, and rhinorrhea. Sneezing and pruritus are initiated pri-

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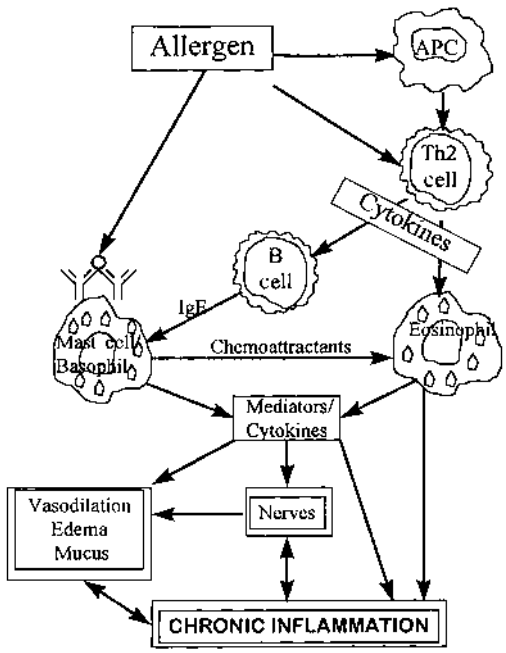

Figure 1 Pathogenesis of allergic rhinitis: Inhaled allergen is processed by antigen-presenting cells (APC), which interact with Th2 lymphocytes causing the release of key cytokines. These released cytokines stimulate B cells to produce and release IgE, which binds to mast cells and basophils in the nasal mucosa. Allergen cross-linking of two IgE molecules on the surface of mast cells/basophils results in the release of mediators and cytokines. Cytokines released from Th2 lymphocytes as well as mediators and cytokines released from mast cells and basophils are important in the growth, differentiation, activation, and chemoattraction of eosinophils into the nasal mucosa. Mediators and cytokines released from mast cells, basophils, and eosinophils cause vasodilation, edema, and mucus secretion and contribute to a chronic inflammatory state. In addition, mediators and cytokines help in the release of neural transmitters and neuropeptides, which contribute to the principal symptoms and inflammatory state of allergic rhinitis.

marily by histamine acting on H1 receptors on sensory nerve endings (14). The effects of histamine on the mucosal vasculature include vasodilation and plasma exudation, which contribute to nasal congestion and rhinorrhea. However, as noted below, neither congestion nor rhinorrhea is mediated primarily by histamine.

Experiments have shown that nasal challenge with ragweed pollen results in increased levels of not only histamine, but also leukotriene C_4 (LTC_4), LTD_4,

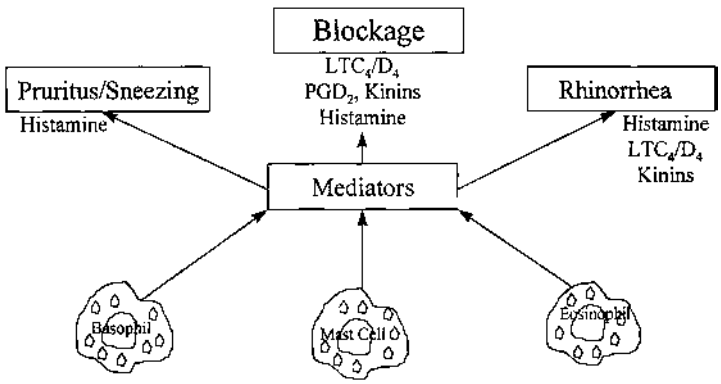

Figure 2 Mediator-induced symptoms of allergic rhinitis. This diagram shows the symptoms induced by the principal mediators released from key inflammatory cells in allergic rhinitis. (Adapted from Ref. 89.)

LTE_4, prostaglandin D_2 (PGD_2), kinins, kininogenase, and tosyl-L-arginine-methyl ester esterase in nasal secretions (15,16). Several mediators contribute to the symptoms of allergic rhinitis, as summarized in Figure 2.

The late phase begins 4–6 hours after allergen exposure and is characterized by nasal congestion, anosmia, mucus secretion, and nasal hyperresponsiveness. Except for PGD_2, the same mediators released during the early phase are recovered during the late phase. This suggests that basophils are important during the late phase because PGD_2 is produced by mast cells but not basophils (17). Indeed, nasal biopsies from allergic rhinitics during symptomatic periods show increased mast cells and basophils (18). Nasal congestion in allergic rhinitis is produced by several mediators including histamine, kinins (through the bradykinin B_2 receptor), leukotrienes, platelet-activating factor (PAF), and PGD_2 (Fig. 2) (19).

The late phase is also characterized by the transendothelial migration and activation of several inflammatory cells. Cell counts in nasal secretions during late phase responses show increases in eosinophils (20), basophils (21), CD_4 T cells, and neutrophils (22). These cells are known to release a wide array of proinflammatory substances. Epithelial and endothelial cells are also involved in the inflammatory process by virtue of their production of key mediators and cytokines. The phenomenon of nasal ''priming'' depends on the accumulation of inflammatory cells and mediators in the nasal mucosa (23). Nasal priming is defined as the need for less antigen to elicit the same degree of symptoms during the peak antigen season versus that required out of the antigen season.

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C. Inflammatory Cell Recruitment

Eosinophil accumulation in the nasal mucosa is an important event in the pathogenesis of allergic rhinitis. Once activated, eosinophils release a variety of mediators, which can contribute to the symptoms of allergic rhinitis. Indeed, the release of LTC_4 by eosinophils leads to both rhinorrhea and nasal obstruction (17,19,20).

The migration of inflammatory cells into the nasal mucosa is mediated through the chemotactic effects of various inflammatory mediators (e.g., eicosanoids, PAF) and cytokines/chemokines (e.g., IL-8, IL-5, RANTES, eotaxin) released by mast cells, epithelial cells, and lymphocytes. Recruitment of leukocytes into the nasal mucosa, however, cannot occur without the proper expression of adhesion molecules on vascular endothelium, epithelium, and leukocytes in response to mediator and cytokine signals. Upregulation of adhesion molecules is very important in allergic inflammation (24,25). In fact, intercellular adhesion molecule-1 (ICAM-1) can be viewed as another hallmark of allergic inflammation (26). ICAM-1 expression on epithelial cells is upregulated during the allergen season (27) and is important in granulocyte recruitment.

Endothelial expression of vascular cell adhesion molecule-1 (VCAM-1) is induced by IL-4 (28). Perennial allergic rhinitis leads to increased expression of ICAM-1 and VCAM-1 in nasal mucosa (29). VCAM-1 upregulation and expression on the endothelium following allergen challenge may contribute to a more selective recruitment of eosinophils into the nasal mucosa because VLA-4, the ligand for VCAM-1, is expressed on eosinophils but not neutrophils (30). VLA-4 is also present on lymphocytes and is an important factor in controlling lymphocyte migration into the airways. Thus, the vasculature of the nasal mucosa is a key element in the control of inflammatory cell chemotactic responses through the expression of important adhesion molecules and the generation of mediators and cytokines.

D. Vasculature

Other critical roles for the vasculature of the nose include heating and humidifying inspired air (31), controlling the expansion (congestion) and shrinkage (decongestion) of the nasal mucosa (32), and regulating edema. Subepithelial and glandular capillaries are fenestrated, thus permitting the exudation of plasma into the submucosa, which in turn supplies the glands with the necessary fluid to produce copious amounts of secretions. Extravasation of plasma occurs during allergic inflammation when gaps are created between endothelial cells by the actions of histamine, bradykinin, PGD_2, LTC_4, LTD_4, and PAF (19,33).

E. Neurogenic Contribution

Sympathetic and parasympathetic efferent nerves innervate the capacitance vessels and regulate vascular tone. Both play an important role in the control of

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blood flow to the nasal mucosa, thereby affecting nasal congestion and plasma exudation (34). Parasympathetic efferents innervate serous and seromucinous glands and, therefore, are critical in the control of nasal secretions. However, sympathetic activity, vasoactive intestinal peptide (VIP), and nitric oxide (NO) can also modulate the activity of submucosal glands (35).

Parasympathetic activity results in vasodilation and increased blood flow to the nose, while sympathetic nerves cause vasoconstriction and decreased blood flow. However, the classical view, which assigned ''opposing'' actions to the sympathetic and parasympathetic systems on their target tissues, has given way to the current view of complex interactions between classical neurotransmitters (acetylcholine and norepinephrine), neuropeptides, target cells, and soluble mediators released by the target cells. The actions of parasympathetic fibers are mediated by acetylcholine, VIP, and NO. Norepinephrine and neuropeptide Y (NPY) are released from sympathetic efferents. NPY is a potent vasoconstrictor of nasal capacitance vessels (36), and it increases the expression of adhesion molecules (37).

Our understanding of allergic inflammation was enhanced following the discovery of the mechanisms of ''neurogenic inflammation'' (35,38). Neurogenic inflammation refers to the neurally mediated reflex stimulation of submucosal gland secretions, plasma transudation, inflammatory cell infiltration, and alterations in epithelial permeability. These events result from activation of sensory fibers, antidromic spread of nerve impulses along sensory axons to other sensory terminals, and release of neuropeptides including substance P, neurokinin A, calcitonin gene–related peptide (CGRP), and gastrin-releasing peptide (GRP) from those terminals. The neuropeptides may directly cause rhinorrhea and nasal congestion and mediate various inflammatory events. Neurogenic influences are thought to play an important role during the late phase of the allergic reaction (14), but the exact mechanisms are still unclear. Exogenous substance P administered intranasally increases vascular permeability, nasal congestion, and chemotaxis of neutrophils and eosinophils in patients with allergic rhinitis (39). Substance P levels increase after antigen challenge, and there is evidence that substance P itself stimulates cytokine expression. CGRP causes proliferation of fibroblasts and epithelium (40). VIP is colocalized in cholinergic and sensory nerve endings. VIP-containing terminals are associated with vasculature, submucosal glands, and the epithelium of the nasal mucosa, all of which possess VIP receptors. Thus, VIP may also play a role in neurogenic inflammation (38).

Parasympathetic reflexes may also regulate mediator release and lymphocyte activity (41). Furthermore, contralateral histamine release and basophil infiltration occur following ipsilateral nasal challenge with allergen (30), an event mediated by neural reflexes.

The role of neuropeptides and sensory and autonomic nerves in allergic rhinitis has been recently reviewed in detail (38,42). It is clear that neurogenic

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pathways play an important role in the acute and chronic symptoms of allergic rhinitis as well as the hyperresponsiveness of the upper airways associated with this disorder.

F. Summary

A summary of pathogenic mechanisms of allergic rhinitis is schematically illustrated in Figure 1. Allergic rhinitis is initiated when allergen cross-links IgE: FcεRI complexes on mast cells (and basophils), causing degranulation and release of multiple mediators. The early phase response is characterized by pruritus, sneezing, rhinorrhea, and nasal congestion resulting from the interaction of these mediators with blood vessels, submucosal glands, and nerve endings. Parasympathetic and other neural reflexes contribute to vasodilation and mucus secretion. Neurogenic inflammation amplifies these processes, and pro-inflammatory mediators including cytokines and chemokines recruit inflammatory cells into the nasal mucosa. The late phase is heralded by the upregulation and expression of leukocyte adhesion molecules in the vasculature. Under the influence of cytokines, chemokines, and other chemoattractants, various leukocytes infiltrate the nasal mucosa where they are activated, releasing more pro-inflammatory mediators. A state of chronic inflammation is established and symptoms of nasal congestion, mucus secretion, anosmia, and nasal hyperresponsiveness predominate at this stage.

III. Diagnosis

A. Symptoms

The diagnosis of allergic rhinitis begins with a careful documentation of the patient's symptoms, their seasonal pattern, and a detailed medical and environmental history. Classic symptoms of allergic rhinitis include paroxysms of sneezing, nasal and palatal pruritus, nasal congestion, and rhinorrhea with or without ocular symptoms (lacrimation, itching, conjunctival swelling). Other common symptoms include postnasal drainage, facial pressure, headaches, nocturnal cough, and snoring.

Malaise and fatigue may be present, sometimes to a debilitating degree. For some patients, fatigue, sedation, difficulty concentrating, and headaches overshadow the nasal symptomatology. Psychosocial problems related to allergic rhinitis include depression, anxiety, irritability, reduced productivity, as well as impaired learning, decision making, and psychomotor speed (43,44). Daytime fatigue can be traced back to sleep disturbance caused by nasal congestion in some patients with perennial allergic rhinitis (45). The severity of rhinitis symptoms may be heightened by other nasal pathology including polyps, septal deviation, sinusitis, and adenoidal hypertrophy.

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B. Physical Findings

Physical findings suggestive of allergic rhinitis include mouth breathing (which can lead to malocclusion), boggy and pale nasal mucosa, clear to mucoid nasal secretions, ''cobblestoning'' (lymphoid hyperplasia) and postnasal drainage along the posterior oropharynx, and conjunctival swelling and injection. Posterior rhinoscopy may be helpful in visualizing structures of the upper airways and differentiating allergic from nonallergic rhinitides (46).

In addition, allergic rhinitics may have a nasal crease across the lower third of the nose (secondary to upward rubbing), allergic shiners (dark circles under the eyes) caused by venous stasis because of constant nasal congestion, Dennie-Morgan lines (extra skin folds on the lower eyelids secondary to conjunctival edema), and certain facial deformities associated with chronic allergic rhinitis such as narrow facies, elevated upper lip, highly arched palate, and retracted lower jaw (47). However, none of these findings are specific for allergic rhinitis.

C. Testing

A nasal smear rich in eosinophils suggests the diagnosis of allergic rhinitis, but it may be only 60% sensitive (48), and it is not specific because eosinophilia is also found in patients with nasal polyps and nonallergic rhinitis with eosinophilia syndrome (NARES) (49). Frequently, however, nasal smears may be used to help differentiate between these forms of rhinitis and infectious rhinitis in which neutrophils predominate.

Total and especially allergen-specific IgE levels are often increased in allergic rhinitis. Skin testing to demonstrate allergen-specific IgE antibodies is the diagnostic test of choice. Alternatively, specific IgE can be measured in the serum using radioallergosorbent testing (RAST) or enzyme-linked immunosorbent assay (ELISA). However, skin testing is more sensitive than these in vitro methods because 25–40% of patients with positive skin tests will have negative RAST to the same allergens (50,51). The converse (a positive in vitro test with a negative skin test) is rare. Thus, except for special clinical situations where skin testing is not possible or the results cannot be interpreted, skin tests are preferable to in vitro tests. Guidelines for the proper application of allergy testing have been published (51).

D. Differential Diagnosis

In the differential diagnosis of allergic rhinitis, one should include the following rhinitides: vasomotor rhinitis, nonallergic rhinitis with eosinophilia syndrome (NARES) (49), atrophic rhinitis (52), occupational rhinitis, medication-induced rhinitis (especially rhinitis medicamentosa due to the overuse of topical sympathomimetics), hormonal rhinitis (pregnancy and hypothyroidism), infectious rhi-

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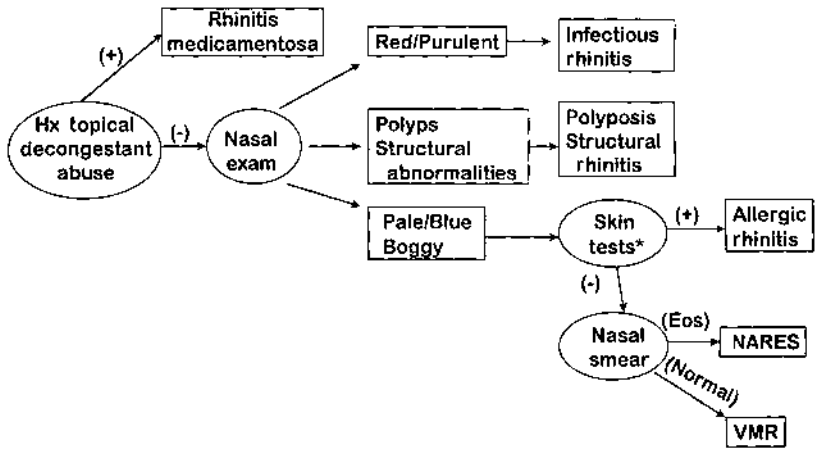

Figure 3 Diagnostic algorithm for the evaluation of rhinitis. *Positive correlation with symptoms. Eos = Eosinophils; NARES = nonallergic rhinitis with eosinophilia; VMR = vasomotor rhinitis.

nosinusitis, and gustatory and food-related rhinitis. Structural and mechanical conditions that mimic rhinitis include deviated nasal septum, hypertrophic turbinates, adenoidal hypertrophy, foreign bodies, and tumors. Inflammatory and immunological diseases such as Wegener's granulomatosis and sarcoid may also cause symptoms of rhinitis. Nasal polyposis predisposes to sinusitis and is associated with asthma, cystic fibrosis (especially in children), and aspirin intolerance. A diagnostic algorithm for some of the more common causes of rhinitis is shown in Figure 3.

IV. Management

A. General Principles

The management of allergic rhinitis includes a stepwise progression through four general therapeutic modalities: (1) avoidance or environmental control; (2) pharmacological therapy; (3) allergen vaccine immunotherapy; and (4) novel immunomodulators such as anti-IgE monoclonal antibodies (e.g., rhuMAb-E25). In formulating a management plan for an individual patient, one must also consider the effects of the disease and the prescribed therapy on frequently occurring comorbid conditions. Left untreated or undertreated, allergic rhinitis has been associated with worsening or the development of other diseases, including otitis media, sinusitis, impairment of smell and taste, and asthma. Indeed, asthma is two to

three times more prevalent in patients with allergic rhinitis, and most adult and pediatric patients with asthma have rhinitis (53). In recently published practice parameters for allergic rhinitis, the panel concluded that the appropriate management of rhinitis may be an important component in effective management of coexisting or complicating respiratory conditions, such as sinusitis, otitis media, and asthma. Furthermore, it was felt that failure to reduce inflammation in the upper airway might lead to suboptimal results in asthma treatment (54).

B. Economic Impact and Quality-of-Life Issues

It is also important to consider the economic impact of allergic rhinitis and coexisting medical problems on health care expenditures (55). When allergic rhinoconjunctivitis was classified as the primary condition, direct expenditures in the United States in 1996 dollars was $1.9 billion. However, when allergic rhinitis was considered a secondary condition coexisting with other airway conditions (e.g., otitis media, sinusitis, asthma, and respiratory tract infections), the cost was $4.0 billion, giving an estimate of $5.9 billion for the overall direct medical expenditures attributable to allergic rhinoconjunctivitis. Children 12 years and younger accounted for $2.3 billion (38%) of these costs. Thus, allergic rhinitis is an expensive disease process because of its direct effects and its contribution to other airway disorders (55).

The health care expenditures cited above do not take into account the additional billions of dollars lost due to the indirect costs of allergic rhinitis. Because the peak prevalence of allergic rhinitis occurs during career- and family-building years, impairments due to this disease significantly impact the work force. Using data from the National (United States) Medical Expenditure Survey conducted in 1987, Malone and colleagues estimated that allergic rhinitis resulted in approximately 811,000 missed workdays, 824,000 missed school days, and 4,230,000 reduced activity days (10). It is likely that the number of workdays lost is much higher due to the effects of childhood absenteeism on the ability of caregivers to report to work.

Impairment of work and school performance has been documented as a result of both the disease and its treatment (7,8,56–58). Allergic rhinitis can lead to sleep-disordered breathing due to nasal congestion, which ultimately contributes to this impairment in work and school performance (59,60). Allergic rhinitis–induced impairment in learning ability in children can be counteracted partially by treatment with nonsedating antihistamines, but is aggravated by treatment with sedating antihistamines (57). In adults, driving performance has been shown to be impaired and work-related injuries increased in allergic rhinitics taking sedating antihistamines (61,62). These data suggest that sedating antihistamines should be used with caution in adults and children who already have decreased quality of life and significant performance impairments.

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C. Environmental Control

Avoidance of allergen-triggering factors, through environmental control measures, is the first line of defense recommended for the management of allergic rhinitis. Environmental control measures may not completely alleviate allergy symptoms, but often symptoms improve and there may be reduced need for pharmacological intervention (63,64).

It is difficult to reduce exposures to pollen and other outdoor allergens. However, pollen-avoidance measures, e.g., staying indoors with windows/doors shut when high pollen counts prevail, are recommended. Mite-avoidance measures include removal of house dust, reduction of indoor humidity, application of allergen-impermeable mattress and pillow covers, and use of chemical agents (e.g., acaricides). Families with a history of atopy should carefully weigh the decision to bring a furred or feathered pet into the household. If pet allergy is a preexisting problem in the household, if at all possible, the pet should be removed from the indoor environment, and, if not possible, at least kept out of the bedroom. Bathing the pet regularly may also be beneficial (63).

D. Pharmacological Intervention

Antihistamines

Antihistamines were discovered in 1937 and have been used in the treatment of allergic rhinitis for over 50 years. Antihistamines compete with histamine for binding to H_1 receptors on blood vessels and smooth muscle. Best results are obtained when antihistamines are given regularly (if needed, on a chronic basis) or prophylactically (prior to allergen exposure) so that the H_1 receptor sites are effectively blocked before histamine release (65).

Antihistamines are divided into three categories: first-, second-, and third-generation antihistamines. They are first-line therapy for the treatment of allergic rhinitis (54).

Oral antihistamines effectively reduce nasal and nonnasal symptoms of allergic rhinitis. Nasal symptoms include itching, sneezing, rhinorrhea, and nasal congestion. Nonnasal symptoms most commonly include eye complaints (e.g., eye redness, tearing, and itching), itchy palate, or itchy ears (54).

Antihistamines are less effective for the treatment of nasal congestion than for other nasal symptoms. Decongestants or topical nasal steroids are typically added to antihistamine therapy to provide symptomatic relief of nasal congestion.

Although oral second-generation antihistamines have a better safety profile than oral first-generation antihistamines, most evaluations show similar efficacy profiles between first- and second-generation antihistamines (54).

Oral antihistamines are readily absorbed and reach peak concentrations within 2–3 hours of dosing. Metabolism of all first-generation and many second-

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generation antihistamines is through the hepatic cytochrome P450 system (54). Clearance rates vary between 2 hours and 10 days. Peak suppression of histamine- or antigen-induced wheal-and-flare reactions occurs 5–7 hours after oral dosing and may persist up to 24–36 hours (longer for hydroxyzine, cetirizine) (65). Astemizole is unique because it binds H_1 receptors with great affinity in comparison to other H_1 blockers and skin test suppression may last up to 6 weeks (54,65).

Intranasal antihistamines are available for treatment of allergic rhinitis symptoms and are appropriate for first-line treatment of allergic rhinitis. Several studies have shown efficacy comparable to oral antihistamines. Onset of action occurs within 3 hours of dosing. Topical administration may impact nasal congestion to a greater degree than oral antihistamines (54).

Two pediatric syrups containing second-generation antihistamines are available for treating children with allergic rhinitis: loratadine (≥6 yr) and cetirizine (≥2 yr) (66).

The adverse effect profile of first-generation antihistamines limits use. First-generation antihistamines are lipophilic and penetrate the blood-brain barrier, where they interact with central nervous system H_1 receptors as well as produce antiserotonin effects. Consequently, first-generation antihistamines are associated with a number of central nervous system (CNS) adverse effects including sedation, slowed reaction time, learning impairment, dystonia, confusion, dizziness, and performance impairment. Because performance impairment may not be perceived, first-generation antihistamines should not be used while flying aircraft or operating heavy machinery (67). Adverse effects are more likely to occur in geriatric adults and patients with liver disease (65).

Second-generation antihistamines do not significantly penetrate the blood-brain barrier and are associated with fewer adverse effects. Because of the improved safety profile, second-generation antihistamines should usually be considered before first-generation antihistamines for the treatment of allergic rhinitis. Of note, though, cetirizine has occasionally been associated with sedation, and terfenadine (withdrawn from U.S. market 2/98) and astemizole have been linked to fatal and near-fatal episodes of cardiac arrhythmias known as torsades de pointes. Concomitant use of macrolide antibiotics (e.g., erythromycin), oral antifungal agents (itraconazole, ketoconazole), and ciprofloxacin and intake of large quantities of grapefruit juice or the presence of liver disease increases the risk for cardiac arrhythmia (54).

Loratadine, a second-generation antihistamine, is well tolerated, nonsedating, and has not been associated with cardiac arrhythmias.

Third-generation antihistamines include metabolites of second-generation antihistamines (e.g., fexofenadine) and topical antihistamines (68).

Fexofenadine is the active metabolite of terfenadine. Its adverse effect profile is improved compared to the parent compound terfenadine, i.e., fexofenadine has not been associated with QT interval prolongation or cardiac arrhythmias

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(e.g., torsades de pointe) (68). Azelastine nasal spray is generally well tolerated but has been associated with bitter taste and sedation in some patients (20 and 11% of patients, respectively) (54).

Older adults are at most risk for adverse effects associated with first-generation antihistamines (68). Because second- and third-generation antihistamines have fewer sedative and anticholinergic adverse effects than first-generation antihistamines, they are generally recommended for the treatment of allergic rhinitis in older patients (66).

Decongestants

There are two categories of decongestants: topical and oral. Decongestants reduce nasal congestion and edema by vasoconstriction of nasal capacitance vessels through stimulation of α_1-adrenoreceptors. They do not improve symptoms of itchy and runny nose or sneezing (54,64). Topical decongestants are either catecholamine derivatives (e.g., phenylephrine) or imidazoline derivatives (e.g., oxymetazoline or xylometazoline). Oral decongestants are α_1-adrenergic agonists (e.g., pseudoephedrine, phenylephrine, and phenyl propanolamine).

Topical and oral decongestants are useful to treat the symptoms of nasal congestion associated with allergic rhinitis. Many combination antihistamine/decongestant preparations are available for the convenience of the patient since decongestants and antihistamines are frequently prescribed together. Combination agents provide greater relief of symptoms but are associated with a higher incidence of adverse effects (70,71).

Topical decongestants do not commonly cause systemic sympathomimetic effects but may lead to rebound congestion (rhinitis medicamentosa) after 5–7 days of therapy.

Oral decongestants are not strongly associated with rhinitis medicamentosa but are associated with systemic adverse effects, e.g., blood pressure elevation, palpitations, tremor, appetite loss, and insomnia. Oral decongestants should be used with caution in patients with cardiac disease (arrhythmias, high blood pressure, coronary heart disease), hyperthyroidism, glaucoma, diabetes, and urinary dysfunction. Pseudoephedrine is less likely to elevate blood pressure than phenyl propanolamine (64).

Corticosteroids

Corticosteroids are very effective for the treatment of allergic rhinitis. Efficacy is related to anti-inflammatory activities including the following: decreased nasal eosinophil, basophil, and goblet cells, decreased mucosal mast cells, inhibition of leukocyte influx into the nasal cavity, inhibition of lymphocyte migration and cytokine release, and inhibition of late-phase allergic response (72,73).

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Oral steroids effectively treat allergic rhinitis, but efficacy is based on systemic absorption, and because of the adverse effect profile, regular use of oral steroids is not justified. A brief treatment course may be warranted to control severe nasal obstruction. When oral steroids are necessary (e.g., cases of severe rhinitis associated with polyposis), short bursts (5–7 days) of short-acting oral steroid (prednisone or methylprednisolone) at doses up to 40 mg/day prednisone in adults may be prescribed. Depot steroid injections are not usually recommended for the following reasons: risk of prolonged adrenal suppression and loss of flexible dosing. Parenteral corticosteroids are not recommended either because of the greater potential for long-term adverse effects. Intraturbinate injections should not be administered because of the link to cavernous vein thrombosis and blindness (54).

Intranasal steroids were developed to avoid adverse effects associated with systemic steroids. Nasal steroids are effective for nasal allergic rhinitis symptoms (sneezing, itching, runny nose, and nasal congestion) (54). In clinical trials, nasal steroids are more effective for control of nasal symptoms than nasal cromolyn sodium or oral antihistamines. However, intranasal steroids do not significantly improve the nonnasal symptoms of allergic rhinitis. Patients frequently take both intranasal steroids and oral antihistamines to control symptoms of seasonal allergic rhinitis. Because onset of action generally occurs a few days after dosing, intranasal steroids are best prescribed prophylactically and used on a regular basis rather than ''as needed'' (65).

The most common adverse effects of intranasal steroids are local irritation (e.g., a burning or stinging sensation) and nasal bleeding. Proper use has not been associated with mucosal atrophy. Long-term use has rarely been associated with nasal septal perforation. Nasal septal perforation may be the result of trauma from the spray velocity combined with vasoconstrictor activity of the steroid. Patients should be instructed to direct the spray away from the nasal septum, and physicians should periodically examine the septum for mucosal erosions, which may precede nasal septal perforation (54).

Intranasal steroids work locally, and systemic side effects are not associated with use in adults when used at recommended doses (65). In children, concerns about growth velocity prompted the U.S. Food and Drug Administration to issue a warning about the safety in children. It is recommended that intranasal steroid use in children be at the lowest effective dose, height measurements be followed regularly, and need for therapy frequently reevaluated (74).

Intranasal Cromolyn Sodium

Intranasal cromolyn sodium is thought to inhibit mast cell degranulation and prevent the allergic reaction leading to the manifestations of allergic rhinitis (54). Clinical studies show pretreatment with intranasal cromolyn sodium 4–7 days

before the seasonal aeroallergen challenge improves seasonal rhinitis symptoms, although less effectively than intranasal steroids. Thus, highly symptomatic patients may require add-on therapies (e.g., antihistamines, decongestants) for symptom control. Use should be continued regularly through the allergy season, but patient compliance may be a problem due to frequent dosing requirements (1–2 sprays every 4 hours).

In situations of limited, anticipated allergen exposure (e.g., animal dander), intranasal cromolyn may provide symptom relief when used shortly before exposure. Intranasal cromolyn sodium is notably safe. Adverse effects are localized to the nasal mucosa and minor. They include sneezing, nasal stinging, burning, irritation, and nose bleeds ($<1\%$). Because of its safety profile, intranasal cromolyn sodium is frequently used in children and pregnant women (54).

Intranasal Anticholinergics

Intranasal anticholinergics effectively reduce rhinorrhea. Ipratropium bromide, oxitropium bromide, tiotpropium bromide, and glycopyrrolate are quaternary structured ammonium muscarinic receptor antagonists, which are poorly absorbed into the systemic circulation. They reduce nasal secretions by influence on submucosal glands innervated by parasympathetic nerves (74). Intranasal anticholinergics are ineffective for control of allergic rhinitis symptoms other than rhinorrhea (74).

With the aqueous formulation, the most frequently reported adverse events are mild, intermittent nosebleeds and nasal dryness. The chlorofluorocarbon-based MDI formulation is associated with high incidence of nasal adverse effects that have limited clinical use of this formulation (54).

Allergen Immunotherapy

Allergen immunotherapy is the intermittent administration of gradually increasing quantities of specific allergen by subcutaneous (SQ) injection to an allergic patient. After the buildup phase, intermittent SQ injections effectively reduce rhinitis symptoms upon reexposure to the specific allergen.

Allergen immunotherapy was first used to treat allergic rhinitis by Noon and Freeman in 1911 (75). Allergen immunotherapy has since been used to treat allergic diseases caused by inhalant allergens. Clinical studies have shown significant treatment benefit for allergic rhinitis induced by grass, tree, and ragweed pollens, molds, house dust mite, and cat (75,76).

Allergen immunotherapy works in part through effects on T cells. There is good evidence that a shift in cytokine profile from Th2 to Th1 occurs in response to allergen immunotherapy. Decreases in IL-4 and increases in IFN-γ were shown in bee venom–sensitive patients undergoing rush immunotherapy. Similar

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changes in cytokine profile were shown in T-cell lines derived from grass- and mite-sensitive patients after immunotherapy (75).

Allergen immunotherapy is indicated for patients who meet the following criteria: (1) IgE-mediated moderate-severe allergic rhinitis; (2) sensitivity correlates with availability of a potent allergenic extract; (3) exposure to an aeroallergen that is significant and unavoidable; (4) symptoms not controlled by pharmacological intervention; (5) pharmacotherapy produces intolerable adverse effects. Immunotherapy is usually not recommended for preschool children and older adults. Immunotherapy is usually not indicated for patients with severe cardiovascular or pulmonary disease and patients using β blockers. Initiation during pregnancy should be avoided, although maintenance therapy is acceptable.

Immunotherapy should be prescribed by physicians trained in allergology. Clinically relevant vaccines must be used because clinical response is specific to the allergen administered in the vaccine. The quality and quantity of allergen vaccine are critical for a successful treatment outcome. The optimal maintenance dose is in the range of 5–20 μg of major allergen per injection for most allergens.

After 1 year of treatment, if the patient has not responded positively, treatment should be stopped. For patients with treatment effect, the optimal duration of treatment is generally within the range of 3–5 years (75).

Of interest are recent reports suggesting that allergen immunotherapy may modify the natural course of the disease. In a recent case-controlled study in which children between the ages of 3 and 6 years were followed for 3 years from baseline, all children who did not receive immunotherapy had new sensitizations, whereas only 50% of the children who received immunotherapy developed new sensitizations (77). The Preventive Asthma Study, conducted in Scandinavia and Germany, also suggests that the development of asthma is less likely to occur in children with allergic rhinitis who are treated with immunotherapy. After 5 years, 58% of control subjects had asthma, compared with 23% of those who received immunotherapy (78). These studies suggest that earlier intervention with immunotherapy in children might be warranted to both decrease sensitization and reduce the development of asthma. Further data are clearly needed to confirm these intriguing results.

The major adverse effect associated with immunotherapy is systemic anaphylaxis. Immunotherapy should only be administered by professionals who can provide emergency treatment and have ready access to resuscitation equipment and medication (antihistamines, epinephrine, steroids). Patients should wait in the clinic at least 20–30 minutes because most serious systemic anaphylactic reactions typically occur within this time frame.

Risk factors for systemic anaphylaxis include (1) errors in dose, (2) unstable asthma in asthmatic patients, (3) highly sensitive patients, (4) use of β blockers, (5) injections from new vials, (6) build-up therapy phase, (7) patients with sea-

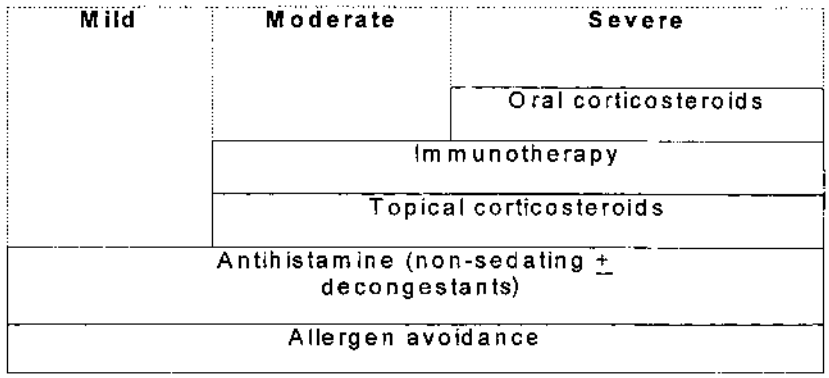

Figure 4 Allergic rhinitis stepwise treatment approach.

sonal exacerbations. Patients who have a systemic anaphylactic reaction should have their allergen immunotherapy regimen reevaluated.

Local reactions may occur at the injection site. These include wheals, delayed swelling, and SQ nodules (associated with aluminum-adsorbed vaccines). Local reactions are painful, and allergen immunotherapy dosage adjustments may be necessary (75).

Stepwise Treatment Approach

Given the wide range of pharmacotherapeutic options available for the treatment of allergic rhinitis, the International Rhinitis Management Working Group developed a stepwise treatment algorithm for prescribing physicians to refer to when developing treatment plans for their patients (63). A simplified stepwise treatment approach for allergic rhinitis is shown in Figure 4.

E. Future Pharmacotherapy

Anti-IgE Approaches

The efficacy of anti-IgE MAb for the treatment of seasonal allergic rhinitis has been tested in several phase II/III clinical studies. Given the fundamental role of IgE in the pathogenesis of seasonal allergic rhinitis (SAR), decreasing total serum IgE in atopic patients was expected to decrease antigen-specific IgE, reduce IgE-mediated symptoms, and improve control of this disease (79).

In adults with mountain cedar SAR, intravenous treatment with a chimeric anti-IgE MAb (CGP 51901) reduced symptoms of SAR during the course of the

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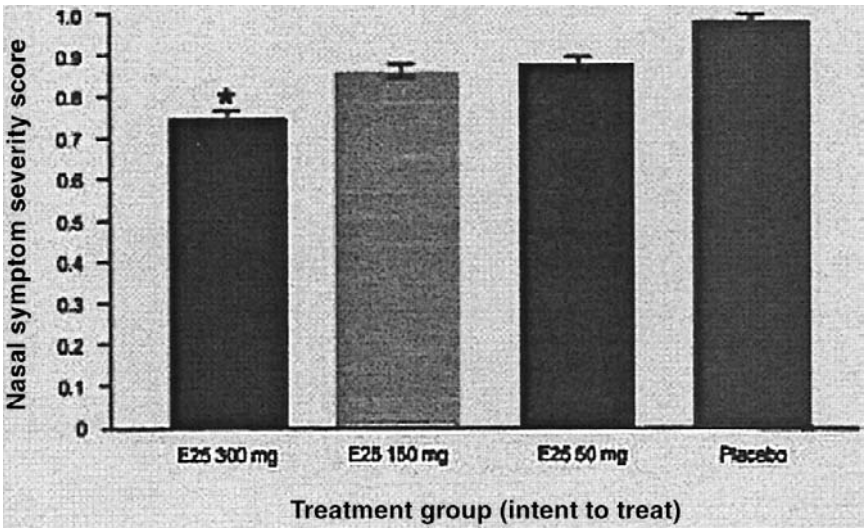

Figure 5 Average daily nasal symptom severity scores for each treatment group (over entire ragweed pollen season). *, $p = 0.002$ versus placebo. (From Ref. 81.)

mountain cedar pollen season. This effect was achieved in patients with IgE levels 85% lower than baseline measurements (80).

The recombinant, humanized anti-IgE MAb (rhuMAb-E25) is in late-stage clinical development for patients with allergic rhinitis. This anti-IgE MAb was been tested in two different models of seasonal pollinosis: ragweed in the central United States and birch in several Scandinavian countries.

rhuMAb-E25 was studied in a large, multicenter, dose-ranging trial to determine the efficacy and safety of three SQ doses of rhuMAb-E25 (50, 150, or 300 mg) for symptom prevention in adolescents and adults with a history of ragweed seasonal allergic rhinitis. Overall results showed that rhuMAb-E25 treatment safely produced dose-dependent improvements in symptoms of SAR, reduced rescue concomitant medication use, and improved rhinitis-specific quality of life (81,82). The 300 mg dose was identified as optimal to study in future clinical trials.

In this dose-ranging study, 536 patients with allergy to ragweed pollen were randomized to receive rhuMAb-E25 or placebo SQ over the 12-week pollen season. Mean daily nasal severity scores in the rhuMAb-E25 300 mg group were superior to placebo over the entire pollen season (0.75 and 0.98 rhuMAb-E25 300mg vs. placebo, respectively; $p = 0.002$) (Fig. 5). Average daily total ocular

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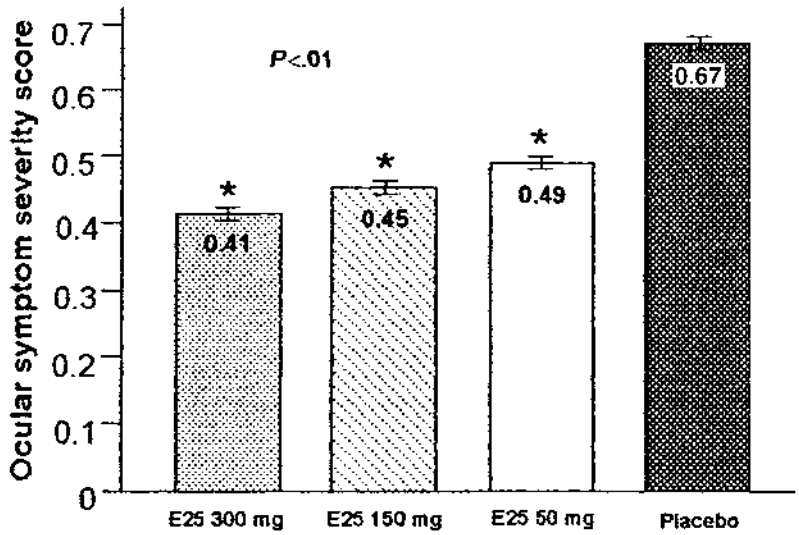

Figure 6 Average daily ocular severity scores for each treatment group (over entire ragweed pollen season). (From Ref. 81.)

severity scores were significantly lower in the 300, 150-, and 50 mg groups vs. placebo (0.41, 0.45, and 0.49 vs. 0.67, respectively; $p = 0.001$, 0.002, and 0.011) (Fig. 6). Regression analysis showed a significant linear dose-response relationship for average daily nasal severity scores (slope for dose -0.001; $p < 0.001$). The proportion of days with rescue antihistamine tablets was significantly lower in the 300 and 150 mg groups vs. placebo (0.12 and 0.13 days vs. 0.21 days, respectively; $p = 0.005$ and 0.012). There was a similar incidence of adverse experiences between rhuMAb-E25 and placebo. Headache, upper respiratory infection, and viral infection were the most frequently reported adverse effects in the rhuMAb-E25 groups. Headache, sinusitis, upper respiratory infection, and viral infection were the most frequently reported adverse effects in the placebo group. Trial drug–related urticaria were reported in two rhuMAb-E25 recipients. There were no trial drug-related serious adverse effects in the rhuMAb-E25 treatment group, and no antibodies to rhuMAb-E25 were detected.

The effect of treatment with rhuMAb-E25 was most recently studied in birch pollen–induced SAR. In this phase III study, results showed that rhuMAb-E25 was safe and effective for the prevention of birch-induced SAR symptoms, with less concomitant medication use and improved quality of life (83,84).

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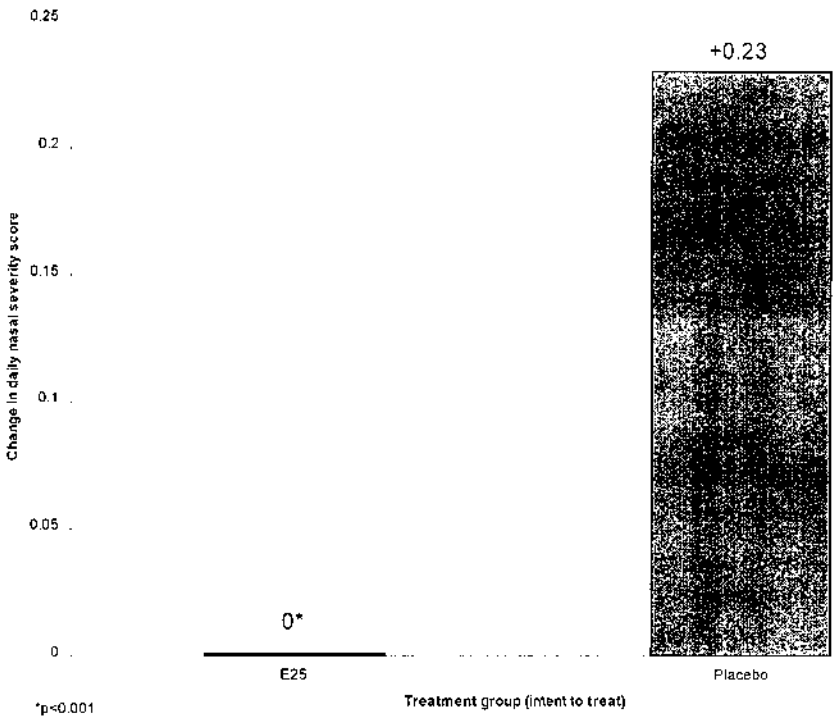

Figure 7 Change from baseline: average daily nasal symptom severity score over entire post-randomization period (which encompassed birch pollen season). (From Ref. 83.)

Two hundred and fifty-one adults were randomized to receive rhuMAb-E25 300 mg or placebo. The average daily nasal symptom severity score in the rhuMAb-E25 group did not change from baseline during the 8-week double-blind treatment period, which encompassed the entire birch pollen season (0.71 and 0.70 baseline and overall on treatment, respectively). In the placebo group, measurements increased from 0.78 at baseline to 0.98 overall on treatment. The between-treatment difference was -0.23 in favor of rhuMAb-E25 ($p < 0.001$) (Fig. 7). The average number of rescue antihistamine tablets taken per day was lower in the rhuMAb-E25 group vs. placebo (difference $= -0.79$ tablets/day; $p < 0.001$). The proportion of days that rescue medication use was taken was almost doubled in the placebo group compared to the rhuMAb-E25 group, 49% vs. 28%, respectively (-0.21 difference; $p < 0.001$). rhuMAb-E25 was well tolerated. There were no anaphylactic/anaphylactoid reactions or other serious drug-related adverse effects. There were no clinically important laboratory findings and no antibodies to rhuMAb-E25 detected.

The results of this clinical study using rhuMAb-E25 to prevent symptoms of birch allergic rhinitis mirrored the result of the preceding study in ragweed-induced SAR. Together, these studies demonstrate and confirm the therapeutic benefit of anti-IgE in SAR. Furthermore, since anti-IgE therapy is not allergen-specific, this therapeutic approach likely will benefit allergic patients with multiple sensitivities.

Others

There is evidence that oral antileukotrienes agents provide benefit for the treatment of allergic rhinitis. Further study is needed before treatment recommendations can be provided (85,86). Rupatadine is a novel orally active dual antagonist of histamine and PAF. This agent is under investigation for the treatment of seasonal and perennial allergic rhinitis (87).

Ramatroban is a thromboxane-prostanoid receptor antagonist. Late-stage clinical studies in patients with allergic rhinitis showed symptom improvement. This compound is in the process of registration in Japan (88).

V. Overall Conclusions

Allergic rhinitis is a common condition. The disease is often trivialized despite high direct expenditures for treatment and high indirect expenditures in terms of missed work days, school days, and reduced activity days. Allergic rhinitis may significantly impact quality of life leading to decreased social and physical functioning (7,8,56–60).

There is a wide range of therapeutic options for the treatment of allergic rhinitis. These therapies, although helpful, may only modestly improve symptoms in patients, especially those at the moderate-to-severe end of the disease severity spectrum.

Over the last 30 years, significant scientific advances have elucidated the biochemical processes that result in acute and chronic allergic inflammatory rhinitis. The basic laboratory work on IgE has translated into the clinical application of anti-IgE MAb as a therapeutic agent for allergic rhinitis. Similar progress has been made with respect to antileukotrienes, PAF inhibitors, a thromboxane-prostanoid receptor antagonist, and inhibitors of important cytokines such as IL4 and IL-5. These advances are expected in the near future to reduce the considerable morbidity that allergic rhinitis patients presently withstand in daily life.

References

1. Sibbald B, Rink E. Epidemiology of seasonal and perennial rhinitis: clinical presentation and medical history. Thorax 1991; 46:895–901.

Marcel Dekker, Inc.
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2. Smith JM. Epidemiology. In: Mygind N, Naclerio RM, eds. Allergic and Non-allergic Rhinitis: Clinical Aspects. Copenhagen: Munksgaard, 1993:15–21.
3. Horak F, Stübner UP. New trends in allergic rhinitis. Allergy Clin Immunol Int 1998; 10:81–87.
4. Nathan RA, Meltzer EO, Selner JC, Storms W. Prevalence of allergic rhinitis in the United States. J Allergy Clin Immunol 1997; 99:S808–S814.
5. Hurst DS. Association of otitis media with effusion and allergy as demonstrated by intradermal skin testing and eosinophil cationic protein levels in both middle ear effusion and mucosal biopsies. Laryngoscope 1996; 106:1128–1137.
6. Newman LJ, Platts-Mills TA, Phillips CD, Hazen KC, Gross CW. Chronic sinusitis: relationship of computed tomographic findings to allergy, asthma and eosinophilia. JAMA 1994; 21:363–367.
7. Bousquet J, Bullinger M, Fayol C, Marquis P, Valentin B, Burtin B. Assessment of quality of life in patients with perennial allergic rhinitis with French version of SF-36 Health Status Questionnaire. J Allergy Clin Immunol 1994; 94(Part 1):182–188.
8. Meltzer EO, Nathan RA, Selner JC, Storms W. Quality of life and rhinitic symptoms: results of a nationwide survey with the SF-36 and RQLQ questionnaires. J Allergy Clin Immunol 1997; 99:S815–819.
9. Fireman P. Treatment of allergic rhinitis: effect on occupation productivity and work force costs. Allergy Asthma Proc 1997; 18:63–67.
10. Malone DC, Lawson KA, Smith DH, Arrighi HM, Battista C. A cost of illness study of allergic rhinitis in the United States. J Allergy Clin Immunol 1997; 99:22–27.
11. McMenamin P. Costs of hay fever in the United States in 1990. Ann Allergy 1994; 73:35–39.
12. Mygind N, Dahl R. The rationale for use of topical corticosteroids in allergic rhinitis. Clin Exp Allergy 1996; 26(suppl 3):2–10.
13. Vercelli D, Gaha RS. Control of immunoglobulin E synthesis. In: Middleton E Jr, Reed CE, Ellis EF, et al., eds. Allergy: Principles and Practice. 5th ed. St. Louis: Mosby, 1998:72–82.
14. Naclerio RM. Pathophysiology of perennial allergic rhinitis. Allergy 1997; 52(suppl 36):7–13.
15. Creticos PS, Adkinson NF, Kagey-Sobotka A, Proud D, Meier HL, Naclerio RM, Lichtenstein LM, Norman PS. Nasal challenge with ragweed pollen in hay fever patients. J Clin Invest 1985; 76:2247–2253.
16. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF Jr, Meyers DA, Norman PS, Lichtenstein LM. Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis 1983; 128:597–602.
17. Naclerio RM, Proud D, Togias AG, Adkinson NF Jr, Meyers DA, Kagey-Sobotka A, Plaut M, Norman PS, Lichtenstein LM. Inflammatory mediators in late antigen-induced rhinitis. N Engl J Med 1985; 313:65–70.
18. Gomez E, Clagne JE, Gatland D, Davies R. Effect of topical corticosteroids on seasonally induced rhinitis increases in nasal mast cells. Br Med J 1988; 296:1572–1573.
19. Howarth PH. Mediators of nasal blockage in allergic rhinitis. Allergy 1997; 52(suppl 40):12–18.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

20. Bascom R, Pipkorn U, Lichtenstein LM, Naclerio RM. The influx of inflammatory cells into nasal washings during late response to antigen challenge: effect of corticosteroid pretreatment. Am Rev Respir Dis 1988; 138:406–412.
21. Bascom R, Wachs M, Naclerio RM, Pipkorn U, Galli SJ, Lichtenstein LM. Basophil influx occurs after nasal antigen challenge: effect of topical corticosteroid pretreatment. J Allergy Clin Immunol 1988; 81:580–589.
22. Varney VA, Jacobson MR, Robinson DS, Irani AM, Schwartz LB, Mackay IS, Kay AB, Durham SR. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Am Rev Respir Dis 1992; 146:170–176.
23. Wachs M, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Observations on the pathogenesis of nasal priming. J Allergy Clin Immunol 1989; 84:492–501.
24. Canonica GW, Ciprandi G, Passalacqua G, Pesce G, Scordamaglia A, Bagnasco M. Molecular events in allergic inflammation: experimental models and possible modulation. Allergy 1997; 52(suppl 34):25–30.
25. Passalacqua G, Ciprandi G, Bagnasco M, Battifora M, Canonica GW. Adhesion molecules and allergy-recent insights. Allergy Clin Immunol Int 1998; 10:23–29.
26. Canonica GW, Ciprandi G, Pesce GP, Buscaglia S, Paolieri F, Bagnasco M. ICAM1 on epithelial cells in allergic subjects: a hallmark of allergic inflammation. Int Arch Allergy Immunol 1995; 107:99–102.
27. Ciprandi G, Pronzato C, Ricca V, Passalacqua G, Bagnasco M, Canonica GM. Allergen specific challenge induces ICAM 1 expression on nasal epithelial cell in allergic subjects: relationships with early and late phase events. Am Rev Respir Crit Care Med 1994; 150:1653–1659.
28. Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, Newman W, Luscinskas FW, Gimbrone MA Jr, McIntyre BW, et al. Interleukin-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. J Immunol 1992; 148:1086–1092.
29. Montefort S, Holgate ST, Howarth PH. Leucocyte-endothelial adhesion molecules and their role in bronchial asthma and allergic rhinitis. Eur Respir J 1993; 6:1044–1054.
30. Naclerio RM, Baroody F. Understanding the inflammatory processes in upper allergic airway disease and asthma. J Allergy Clin Immunol 1998; 101(No 2, Part 2): S345–S351.
31. Widdicombe JG. The airway vasculature. Exp Physiol 1993; 78:433–452.
32. Djukanovic R, Wilson SJ, Howarth PH. Pathology of rhinitis and bronchial asthma. Clin Exp Allergy 1996; 26(suppl 3):44–51.
33. Rajakulasingam K, Polosa R, Church MK, Holgate ST, Howarth PA. Kinins and rhinitis. Clin Exp Allergy 1992; 22:734–740.
34. Baroody FM, Ford S, Lichtenstein LM, Kagey-Sobotka A, Naclerio RM. Physiologic responses and histamine release after nasal antigen challenge: effect of atropine. Am J Respir Crit Care Med 1994; 149:1457–1465.
35. Widdicombe JG. Neuroregulation of the nose and bronchi. Clin Exp Allergy 1996; 26(suppl 3):32–35.
36. Baraniuk JN, Silver PB, Kaliner MA, Barnes PJ. Neuropeptide Y is a vasoconstrictor in human nasal mucosa. J Appl Physiol 1992; 73:1867–1872.

MARCEL DEKKER, INC.
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37. Sung CP, Arleth AJ, Feverstein GZ. Neuropeptide Y upregulates the adhesiveness of human endothelial cells for leukocytes. Clin Res 1991; 68:314–318.
38. Casale TB, Baraniuk JN. Neurogenic control of inflammation and airway function. In: Middleton E Jr, Reed CE, Ellis EF, et al., eds. Allergy: Principles and Practice. 5th ed. St. Louis: Mosby, 1998:183–203.
39. Braunstein G, Fajac I, Lacronique J, Forssard N. Clinical and inflammatory responses to exogenous tachykinins in allergic rhinitis. Am Rev Respir Dis 1991; 144: 630–635.
40. Baraniuk JN, Lundgren JD, Goff J, Mullol J, Castellino S, Merida M, Shelhamer JH, Kaliner MA. Calcitonin gene-related peptide in human nasal mucosa. Am J Physiol 1990; 258:L81–L88.
41. Wagenmann M, Baroody FM, Cheng CC, Kagey-Sobotka A, Lichtenstein LM, Naclerio RM. Bilateral increases in histamine after unilateral nasal allergen challenge. Am J Respir Crit Care Med 1997; 155:426–431.
42. Baraniuk JN. Neuropeptides. Am J Rhinol 1998; 12:9–16.
43. Juniper EF, Guyatt GH. Development and testing of a new measure of health status for clinical trials in rhinoconjunctivitis. Clin Exp Allergy 1991; 21:77–83.
44. Marshall PS, Colon EA. Effects of allergy season on mood and cognitive function. Ann Allergy 1993; 71:251–258.
45. Craig TJ, Teets S, Lehman EB, Chinchilli VM, Zwillich C. Nasal congestion secondary to allergic rhinitis as a cause of sleep disturbance and daytime fatigue and the response to topical nasal corticosteroids. J Allergy Clin Immunol 1998; 101:633–637.
46. Georgitis JW, Druce HM, Goldstein S, Meltzer EO, Okuda M, Selner JC, Schumacher MJ. Rhinopharyngolaryngoscopy. J Allergy Clin Immunol 1993; 91:961–962.
47. Marks MB. Physical signs of allergy on the respiratory tract in children. Ann Allergy 1969; 25:310.
48. Ledford DK, Lockey RF. Allergy rhinitis: offering relief this season. J Respir Dis 1998; 19:647–666.
49. Jacobs RL, Freedman PM, Boswell RN. Non-allergic rhinitis with eosinophilia (NARES syndrome): clinical and immunological presentation. J Allergy Clin Immunol 1981; 67:253–262.
50. Ownby DR. Clinical significance of immunoglobulin E. In: Middleton E Jr, Reed CE, Ellis EF, et al, eds. Allergy: Principles and Practice. 5th ed. St. Louis: Mosby, 1998:770–782.
51. Joint Task Force on Practice Parameters. Practice parameters for allergy diagnostic testing. Ann Allergy Asthma Immunol 1995; 75(Dec, Part II):A1–A5, 543–625.
52. Goodman WS, deSouza FM. Atrophic rhinitis. In: English GM, ed. Otolaryngology, Vol 2. Philadelphia: JB Lippincott, 1987:1–11.
53. Casale, TB. Immunobioloigy of asthma and rhinitis: pathogenic factors and therapeutic options. American Thoracic Society Workshop Summary, Montreal, Canada, June, 1997. New York: American Lung Association, 1999.
54. Diagnosis and management of rhinitis: parameter documents of the Joint Task Force on Practice Parameters in Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 1998; 81(Part II):463–518.

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55. Ray NF, Baraniuk JN, Thamer M, Rinehart CS, Gergen PJ, Kaliner M, Josephs S, Pung YH. Direct expenditures for the treatment of allergic rhinoconjunctivitis in 1996, including the contributions of related airway illnesses. J Allergy Clin Immunol 1999; 103:401–407.
56. The impact of allergic rhinitis on quality of life and other airway diseases. Summary of a European conference. Allergy 1998; 53(suppl 43):1–31.
57. Vuurman EFPM, Van Veggel LMA, Uiterwijk MMC, Leutner D, O'Hanlon JF. Seasonal allergic rhinitis and antihistamine effects on children's learning. Ann Allergy 1993; 71:121–126.
58. Juniper EF, Guyatt GH, Dolovich J. Assessment of quality of life in adolescents with allergic rhinoconjunctivitis: development and testing of a questionnaire for clinical trials. J Allergy Clin Immunol 1994; 93:413–423.
59. Scharf MB, Cohen AP. Diagnostic and treatment implications of nasal obstruction in snoring and obstructive sleep apnea. Ann Allergy Asthma Immunol 1998; 81: 279–287.
60. Young T, Finn L, Kim H. Nasal obstruction as a risk factor for sleep-disordered breathing. J Allergy Clin Immunol 1997; 99:S757–S762.
61. Warren R, Simpson H, Hilchie J, et al. Drugs detected in fatally injured drivers in the province of Ontario. In: Goldberg L, ed. Alcohol, Drugs and Traffic Safety. Vol 1. Stockholm: Almquist and Wksell, 1981:203–217.
62. Gilmore TM, Alexander BH, Mueller BA, Rivara FP. Occupational injuries and medication use. Am J Ind Med 1996; 30:234–239.
63. International Rhinitis Management Working Group. International consensus report on the diagnosis and management of rhinitis. Allergy 1994; 49(suppl 19):5–34.
64. Meltzer EO, Schatz M, Zeiger RS. Allergic and nonallergic rhinitis. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, eds. Allergy Principles and Practice. 3rd ed. St. Louis: CV Mosby Company, 1988:1253–1289.
65. Rachelefsky, GS. Pharmacologic management of allergic rhinitis. J Allergy Clin Immunol 1998; 101(2)(suppl):S367–S369.
66. Day J. Pros and cons of the use of antihistamines in managing allergic rhinitis. J Allergy Clin Immunol 1999; 103(3)(suppl):S395–S399.
67. Simons FER. Antihistamines. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, eds. Allergy Principles and Practices. 5th ed. St. Louis: CV Mosby Company, 1998:612–637.
68. DeBuske L. New concepts in pharmacodynamics of antihistamine therapies for allergic disease: risk/benefit assessment. IRINE Optimal Paradigms for Allergy and Asthma Managed Care: Quality of Life, Outcomes and Risk/Benefits Assessments, Short Hills, NJ, April 10, 1999.
69. Lumry W. Treatment paradigms for allergic rhinitis. IRINE Optimal Paradigms for Allergy and Asthma Managed Care: Quality of Life, Outcome and Risks/Benefits Assessments, Short Hills, NJ, April 10, 1999.
70. Meran A, Morse J, Gibbs TG. A cross-over comparison of acrivastine, pseudoephedrine and their combination in seasonal allergic rhinitis. Rhinology 1990; 28:33–40.
71. Henauer S, Seppey M, Huguenot C, Pécoud A. Effects of terfenadine and pseudoephedrine, alone and in combination in a nasal provocation test and in perennial rhinitis. Eur J Clin Pharmacol 1991; 41:321–324.

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72. Meltzer EO. The pharmacological basis for the treatment of perennial allergic rhinitis and non-allergic rhinitis with topical corticosteroids. Allergy 1997; 52(suppl 36): 33–40.
73. Schleimer RP. Glucocorticosteroids: their mechanisms of action and use in allergic diseases. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, eds. Allergy Principles and Practice. 3rd ed. St. Louis: CV Mosby Company, 1988: 739–765.
74. Segura T, Casale T. Allergic rhinitis: basic pathophysiology and therapeutic strategies. Can J Allergy Clin Immunol. 1999; 4(7):318–330.
75. Bousquet J, Lockey RF, Malling H-J. WHO position paper allergen immunotherapy: therapeutic vaccines for allergic diseases. Allergy 1998; 53(44)(suppl):1–44.
76. Grammer LC, Shaughnessy MA. Principles of immunologic management of allergic diseases due to extrinsic antigens. In: Patterson R, Grammer LC, Greenberger PA, eds. Allergic Diseases. 5th ed. Philadelphia: Lippincott-Raven, 1997:209–221.
77. Des Roches A, Paradis L, Ménardo J-L, et al. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. J Allergy Clin Immunol 1997; 99:450–453.
78. Jacobsen L, Dreborg S, Ferduosi HA, et al. Prevention of asthma by specific immunotherapy (the PAT-study). Five year follow up. European Academy of Allergy and Clinical Immunology Annual Meeting, Birmingham, United Kingdom, 1998. Abstract.
79. Heusser C, Jardieu P. Therapeutic potential of anti-IgE antibodies. Curr Opin Immunol 1997; 9:805–814.
80. Racine-Poon A, Botta L, Chang TW, Davis FM, Gygax D, Liou RS, Rohane PW, Staehlin T, van Steijn AMP, Frank W. Efficacy, pharmacodynamics and pharmacokinetics of CGP51901, an anti-immunoglobulin E chimeric monoclonal antibody, in patients with seasonal allergic rhinitis. Clin Pharmacol Ther 1997; 62:675–690.
81. Casale T, Condemi J, Miller SD, Fick R, McAlary M, Fowler-Taylor A, Gupta N, Rohane PW. rhuMAb-E25 in the treatment of seasonal allergic rhinitis (abstr). Ann Allergy Asthma Immunol 1999; 82:75.
82. Nayak A, LaForce C, Rowe M, Watrous M, Fick R, McAlary M, Gupta N, Fowler-Taylor A, Rohane P. rhu-MAb-E25 improves quality of life in patients with seasonal allergic rhinitis (abstr). J Allergy Clin Immunol 1999; 103:49, part 2.
83. Ädelroth E, Sandström T, Rak S, Rosenhall L, Zetterström O, Aasand OG, Haahtela T, Byrne A, Thirlwell J, Della Cioppa G, Rohane P, Fick R. rhuMAb-E25 treatment controls the symptoms of birch pollen induced seasonal allergic rhinitis and reduces the requirement for rescue medication (abstr). Allergy 1999; 54(suppl 50):7.
84. Ädelroth E, Sabina R, Haahtela T, Aasand G, Rosenhall L, Zetterström O, Byrne A, Champain K, Thirlwell J, Della-Cioppa G, Sandstrom T. Recombinant humanized MAb-E25, an anti-IgE MAb, in birch pollen-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2000; 106(2):253–259.
85. Donnelly AL, Glass M, Minkewitz MC, Casale TB. The leukotriene D4-receptor antagonist, ICI204, 219, relieves symptom of acute seasonal allergic rhinitis. Am J Respir Crit Care Med 1995; 151:1734–1739.
86. Dahlén B, Nizamkowska E, Szczeklik A, Zetterström O, Bochenek G, Kumlin M, Mastalerz L, Pinis G, Swanson LJ, Boodhoo TI, Wright S, Dubé LM, Dahlén S-E.

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Benefits from adding the 5-lipoxgenase inhibitor Zileuton to conventional therapy in aspirin intolerant asthmatics. Am J Respir Crit Care Med 1998; 157:1187–1194.
87. Merlos M, Giral M, Balsa D, Ferrando R, Queralt M, Puigdemont A, Garcia-Rafanell J, Forn J. Rupatadine, a new potent, orally active dual antagonist of histamine and platelet-activating factor (PAF). J Pharm Exp Ther 1997; 280:114–121.
88. Baba S, Unno T, Takasaka T, Baba K, Sakakura Y. A late phase II, dose-finding study of ramatroban (BAY u 3405) in patients with perennial allergic rhinitis. Rinsho Iyaku 1996; 12:2561–2591 [Japanese].
89. Howarth PH. The cellular basis for allergic rhinitis. Allergy 1995; 50(suppl 23):6–10.

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16

Anti-IgE and Allergic Skin Diseases

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I. Introduction

This chapter will review our current knowledge about the pathogenesis of allergic skin disorders, in particular atopic dermatitis (AD) and urticaria. Anti-IgE therapy has not been systematically studied in these skin diseases. Therefore we will discuss the role of IgE in their pathogenesis and provide a scientific rationale for use of anti-IgE treatment in these diseases. Recent studies indicate that the IgE molecule has a multifunctional role in these diseases, acting as both a target and a key player in the elicitation of allergen-induced immediate hypersensitivity reactions and cell-mediated responses.

II. Atopic Dermatitis

A. Clinical Presentation

AD is a chronic inflammatory skin disorder commonly associated with allergic rhinitis and asthma (1). The diagnosis of AD is based on clinical criteria first described by Hanifin and Rajka (2). The major features include pruritus, typical morphology and distribution of the skin lesions, and a chronic relapsing course.

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In infants, AD primarily involves the face, scalp, and extensor surfaces of the extremities, whereas in older children the predominant location of skin lesions are the flexural folds of the extremities. The course of AD may be acute with intense pruritus and papulation. Chronic skin lesions are associated predominantly with thickened skin, lichenification, and fibrotic papules. Dry skin is commonly seen in all patients with AD and contributes to the characteristic pruritus that plagues patients with this disease.

AD should be distinguished from seborrheic dermatitis, where pruritus is absent or mild. Allergic contact dermatitis, which can become generalized, is morphologically difficult to distinguish from AD. Some immunodeficiencies may be associated with eczema, like Wiskott-Aldrich syndrome, severe combined immunodeficiency, or hyper IgE syndrome. Most of the time, AD begins early in life. In half of children with AD, the disease was present before one year of age, and in two thirds before the age of 7 years. The majority of children may outgrow AD by adolescence, but in some patients the disease relapses in adulthood. The actual prognosis of AD is a matter of active investigation. Recent studies suggest that AD may persist in up to 91% of patients, although it frequently becomes less severe (3). The prevalence of this disease has increased considerably in the last few decades affecting over 16% of children in certain countries (4,5). However, there is considerable worldwide variation in the prevalence of AD, both within and between countries inhabited by similar ethnic groups, suggesting that environmental factors are critical in determining disease expression (5).

B. Immunopathogenesis of AD

Although the pathogenesis of AD is poorly understood, the concept that AD has an immunological basis is supported by the observation that patients with primary T-cell immunodeficiency disorders frequently have elevated serum IgE levels and eczematoid skin lesions indistinguishable from AD (6). In patients with Wiskott-Aldrich syndrome, successful bone marrow transplantation is associated with correction of the immunological defect and clearing of their eczema (7). Furthermore, nonatopic recipients receiving bone marrow transplants from atopic donors can develop atopic symptoms following successful engraftment (8). These data suggest that AD is mediated by a bone marrow–derived cell.

Peripheral Blood Studies

Most patients with AD have elevated serum IgE levels and increased numbers of circulating eosinophils (1). These laboratory findings are thought to result from the increased expression of T helper type 2 (Th2)-type cytokines, i.e., IL-4, IL-5, and IL-13 with a concomitant decrease in interferon-gamma (IFN-γ) expression. IL-4 and IL-13 are known to act as IgE isotype-specific switch factors and induce the expression of VCAM-1, an adhesion molecule involved in the

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migration of mononuclear cells and eosinophils into sites of tissue inflammation (9). IL-4 also causes marked inhibition of IFN-γ production and downregulates the differentiation of T helper type 1 (Th1) cells. IL-5 promotes the differentiation, vascular endothelial adhesion, and survival of eosinophils. In contrast, IFN-γ inhibits IgE synthesis as well as the proliferation of Th2 lymphocytes.

In this regard, peripheral blood lymphocytes from AD patients have been reported to secrete increased amounts of IL-4 and express abnormally high levels of IL-4 receptor as well as increased IL-13 (10,11). PBMC from AD patients have also been found to have a decreased capacity to produce IFN-γ (12,13). A significant inverse correlation between IFN-γ generation and IgE serum concentrations in AD has been reported (12). There have also been a number of studies demonstrating an increased frequency of allergen-specific T cells producing increased IL-4, IL-5, and IL-13 but little IFN-γ in the peripheral blood of patients with AD (14). Peripheral T cells expressing the skin homing receptor cutaneous lymphoid antigen (CLA) have also been reported to express increased levels of Th2 cytokines (11,15) (Table 1).

Immunohistology of Atopic Dermatitis

The histological features of AD depend on the acuity of the skin lesion (16,17). *Unaffected* or clinically normal skin of AD patients demonstrates mild hyperkeratosis, epidermal hyperplasia, and a sparse perivascular T-cell infiltrate. Acute lesions are characterized by marked intercellular edema (spongiosis) of the epidermis. A sparse epidermal infiltrate consisting primarily of T lymphocytes is frequently observed. In the dermis, there is a marked perivenular inflammatory cell infiltrate consisting predominantly of memory T lymphocytes bearing the CLA skin homing receptor, CD3, CD4, and CD45 RO (suggesting previous encounter with antigen) with occasional monocyte-macrophages (17,18). Eosinophils, basophils, and neutrophils are rarely present in the acute lesion. Mast cells, in various stages of degranulation, are present in normal numbers.

Table 1 Immunological Abnormalities in AD

Increased IgE synthesis
Increased expression of CD23
Increased levels of IL-4 receptor on lymphocytes
Increased production of increased IL-4, IL-5, and IL-13, but little IFN-γ, by allergen-specific T cells
Increased production of Th2 cytokines by peripheral T cells expressing CLA
Increased eosinophil cationic protein levels in the serum
Increased basophil histamine release in vitro

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In *chronic* lichenified lesions, the epidermis is hyperplastic with elongation of the rete ridges, prominent hyperkeratosis, and minimal spongiosis. There is an increased number of IgE-bearing Langerhans cells in the epidermis, and macrophages dominate the dermal mononuclear cell infiltrate. The number of mast cells are increased in number but are fully granulated. Increased numbers of eosinophils are observed in chronic AD skin lesions. In support of a functional role of eosinophils in chronic AD lesions, Leiferman et al. have found that eosinophil-derived extracellular major basic protein (MBP) can be detected by immunofluorescence in a fibrillar pattern associated with the distribution of elastic fibers throughout the upper dermis (19). Extracellular MBP deposition was much more extensive in the involved areas than the unaffected areas of skin. Although the role of eosinophils in the pathogenesis of AD is not completely understood, it is thought to contribute to tissue injury in AD through the production of reactive oxygen intermediates and release of cytotoxic granules. Eosinophil cationic protein is elevated in AD sera and correlates with disease severity, providing further evidence for the involvement of eosinophils in AD (20).

Cytokine Expression in AD Skin

Recent studies indicate that both Th2- and Th1-type cytokines contribute to the pathogenesis of skin inflammation in AD with the relative contribution of each cytokine type critically dependent on the duration of the skin lesion. In studies by Hamid et al. (21,22) in situ hybridization was used to investigate the expression of IL-4, IL-5, and IFN-γ mRNA in skin biopsies from clinically normal (unaffected), acute (erythematous AD lesions of <3 days duration), and chronic (>2 weeks duration) skin lesions of patients with AD. As compared with normal skin, unaffected skin of patients with AD had a significant increase in number of cells expressing IL-4 and IL-13, but not IL-5 or IFN-γ mRNA. Acute and chronic skin lesions, when compared to normal skin or unaffected skin of AD patients, had significantly greater numbers of cells that were positive for IL-4, IL-5, and IL-13 mRNA. However, acute AD or unaffected AD skin did not contain significant numbers of IFN-γ mRNA–expressing cells.

As compared with acute AD, chronic AD skin lesions had significantly fewer IL-4 and IL-13 mRNA–expressing cells, but increased numbers of IL-5 and IFN-γ mRNA–expressing cells. Thus, acute skin inflammation in AD is associated with a predominance of IL-4 and IL-13 expression, whereas maintenance of chronic inflammation is associated with increased IL-5 and IFN-γ expression accompanied by the infiltration of eosinophils and macrophages.

There is also overexpression of IL-16 in acute skin lesions, as well as IL-12 and granulocyte macrophage colony-stimulating factor (GM-CSF) overexpression in chronic AD lesions (22–24). IL-16 may promote the infiltration of CD4+ T cells into acute lesions, and GM-CSF is likely to enhance cell survival

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of eosinophils and macrophages in chronic skin lesions. The increased expression of IL-12 in chronic AD skin lesions is of interest in that cytokine plays a key role in Th1 cell development and its expression in eosinophils and/or macrophages is thought to initiate the switch to Th1 cell development in chronic AD skin lesions (25). The C-C chemokines RANTES, monocyte chemotactic protein-3, and eotaxin have also been found to be increased in AD skin lesions and likely contribute to the chemotaxis of eosinophils into the skin (26).

The functional significance of these cytokine changes in acute vs. chronic AD skin lesions is supported by in vivo studies of cytokine receptor mRNA expression (27). These studies have demonstrated that acute AD is associated with a high expression of IL-4 receptor, whereas IL-5 receptor and GM-CSF receptor mRNA are predominantly increased in chronic AD. These findings support the biphasic role of IL-4, IL-5, and GM-CSF in the pathogenesis of AD skin inflammation.

This biphasic pattern of T-cell activation has also been well demonstrated in studies on skin biopsies of atopy patch-test reaction sites (25). At early time points, e.g., 24 hours after allergen application, increased expression of IL-4 mRNA and protein is observed, after which IL-4 expression declines to baseline levels. In contrast, IFN-γ mRNA expression is not detected in 24-hour patch-test lesions but is strongly overexpressed at the 48–72 hour time points. This is consistent with studies demonstrating allergen-specific Th2 cell clones derived from early time points of evolving atopy patch test reactions, whereas the majority of allergen-specific T-cell clones derived from later patch-test sites (>48 hours) exhibit a Th1- or Th0-type cytokine profile. Interestingly the increased expression of IFN-γ mRNA in atopy patch test lesions is preceded by a peak of IL-12 expression implicating IL-12 in the development of the Th1-type response. This increased IL-12 expression coincides with the infiltration of macrophages and eosinophils, both cell types known to express IL-12. These observations led to the proposal that initiation of AD is driven by allergen-induced activation of Th2-type cells, whereas the chronic inflammatory response is dominated by a Th1-type response driven by the subsequent infiltration of IL-12–expressing eosinophils and macrophages, which accompanies the initial Th2 response. Of note, IFN-γ has been found to potentiate the activity and survival of eosinophils (28). Thus Th1 cytokines may promote Th2-type inflammation in AD.

C. Role of IgE in AD

Several observations suggest that IgE plays an important role in AD (29,30): the majority of the patients with AD have a family history of atopy, more than 80% of them have increased serum IgE (Table 2), and it has been demonstrated that allergens can exacerbate the skin lesions. Approximately 85% of AD patients have positive immediate skin tests or RAST for specific IgE to a variety of food

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Table 2 Evidence for IgE Involvement in AD

Positive family history for atopy in the majority of patients
Increased serum IgE in 80% of the patients
Respiratory allergies develop later in life in a majority of children with AD
Positive immediate skin tests or serum-specific IgE for allergens
Immediate exacerbation of skin inflammation by exposure to allergenic food in 30–60% of AD children
Immediate exacerbation of skin inflammation by exposure to inhalants in older children
Presence of IgE against staphylococcal toxins in AD patients
Evidence of basophil degranulation by toxins in patients with antitoxin IgE
IgE-bearing Langerhans cells enhances allergen presentation in AD
Presence of IgE to autoallergens in the serum of patients with AD

and inhalant allergens (31,32). A majority of children with AD during the first years of life are at risk to develop symptoms of respiratory allergy, like asthma or allergic rhinitis, later in life. Therefore, AD is an interesting model to investigate the potential use of anti-IgE for early intervention in allergic diseases because it is one of the first manifestations of atopy. Indeed, early exposure to allergens has been shown to correlate well with the risk of acquiring clinical symptoms of allergy (33). Thus, the first year of life is critical for subsequent sensitization to allergens, as demonstrated by studies on early exposure to house dust mites and subsequent development of asthma. In early childhood, the presence of food allergen–specific IgE is common in both atopic and normal infants, peaking prior to one year of age and declining to undetectable levels by the age of 2 or 3 years. However, only a small percentage of these individuals will have a persistent IgE response to food allergens (34).

Immediate Hypersensitivity Reactions

A number of studies have demonstrated the role of food allergens in the exacerbation of AD (reviewed in Refs. 35 and 36). Thirty to 60% of AD children have acute exacerbations of skin symptoms by foods and, in appropriately diagnosed food-sensitive patients, allergen-elimination diet can significantly improve the skin lesions. The role of food hypersensitivities in AD exacerbations has been documented by double-blind placebo-controlled food challenges, where skin reactions develop within 2 hours of challenge and in some patients 6–8 hours later. Of note, plasma histamine levels rise in children following positive oral food challenges but not after placebo challenges (37). Sampson et al. (38) also reported higher rates of spontaneous histamine release from basophils in patients with AD and food hypersensitivity compared with controls. The increased spontaneous

basophil histamine release was dependent on continued ingestion of offending food allergens. Mononuclear cells from patients with food allergy produced an IgE-dependent histamine-releasing factor in vitro that induced histamine release from basophils of other food-sensitive patients, but not from normal controls. Importantly, repeated ingestion of foods to which a patient is sensitized appears to contribute to the development of AD skin lesions.

There is also increasing evidence that inhalant allergens can contribute to the exacerbations of AD, particularly in older children and adults. In children over the age of 3 years, IgE sensitization to aeroallergens has a prevalence of over 90% (39). Involvement of aeroallergens in the exacerbation of AD is supported experimentally by the observation that patch testing with house dust mite allergen extract results in an eczematous rash (40). Positive patch tests in AD patients have been found for other allergens, like weeds, animal dander, and molds (41). Dust mite control has been found to significantly reduce the skin severity of AD (42).

Microbial products from *Staphylococcus aureus* or fungi may also be an important immunological trigger in chronic AD. Superficial fungal infections appear to occur more frequently in atopic individuals. Recently there has been considerable interest in *Malassezia furfur* (also known as *Pityrosporum ovale* or *P. orbiculare*) as a pathogen in AD. IgE antibodies against *M. furfur* is commonly found in AD patients and most frequently in patients with head and neck dermatitis (43). Positive allergen patch test reactions to this yeast have been demonstrated (44). The potential importance of *M. furfur* as well as other dermatophyte infections is further supported by the reduction of AD skin severity in patients following treatment with antifungal agents such as ketoconazole (45,46).

There has been a considerable amount of interest in the relationship between AD and staphylococcal colonization, which is observed in more than 90% of patients with AD (47,48). The importance of *S. aureus* in AD is supported by the observation that not only patients with impetiginized AD, but also AD patients without superinfection show clinical response to combined treatment with anti-staphylococcal antibiotics and topical corticosteroids (49). Recent studies suggest that one strategy by which *S. aureus* exacerbates or maintains skin inflammation in AD is by secreting a group of toxins known to act as superantigens, which stimulate marked activation of T cells and macrophages (50–52). Several lines of investigation support a role for superantigens in AD. First, over half of AD patients have *S. aureus* cultured from their skin that secrete superantigens such as enterotoxins A, B, and toxic shock syndrome toxin-1 (TSST-1). Second, most AD patients make specific IgE antibodies directed against the staphylococcal toxins found on their skin (50). Basophils from patients with antitoxin IgE release histamine on exposure to the relevant toxin, but not in response to toxins to which they had no specific IgE. Third, a correlation has been found between the presence

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of IgE antisuperantigens and severity of AD (52). Utilizing a humanized murine model of skin inflammation, *S. aureus* toxin plus allergen was shown to have an additive effect in inducing cutaneous inflammation (53).

Cell-Mediated Reactions

The histological features of AD are most consistent with a T-cell–mediated response. IgE appears to induce or enhance allergic T-cell responses via two mechanisms. First, 3–4 hours after the immediate hypersensitivity reaction begins to subside, there is an onset of an IgE-dependent late-phase response (LPR) characterized initially by expression of leukocyte adhesion molecules on postcapillary venular endothelium, followed by the infiltration of eosinophils, neutrophils, and mononuclear cells into the inflamed area. Granulocytes reach their maximum cell accumulation at 6–8 hours, and by 24–48 hours after onset of the reaction, the cellular infiltrate consists predominantly of mononuclear cells. The cellular infiltrate in allergen-induced LPRs express increased mRNA for IL-3, IL-4, IL-5, and GM-CSF but not IFN-γ (54). These results suggest that the T cells infiltrating into the allergen-induced LPR, similar to allergen-specific T cells grown from AD skin lesions, are Th2-like cells.

Second, Langerhans cells and macrophages infiltrating into the AD skin lesion bear IgE antibody on the cell surface (55,56). Binding of IgE to Langerhans cells occurs primarily via high-affinity IgE receptors (57). Macrophages can express low-affinity IgE receptors (CD23) in response to IL-4 or GM-CSF (58). Allergens have been demonstrated to activate IgE-bearing macrophages in an IgE-dependent manner with the formation of leukotrienes, PAF, IL-1, and TNF (59). Furthermore, IgE-bearing Langerhans cells in AD skin appear to play an important role in cutaneous allergen presentation to Th2 cells. In this regard, IgE-bearing Langerhans cells from AD skin lesions, but not Langerhans cells that lack surface IgE, are capable of presenting house dust mite allergen to T cells (60). These results suggest that cell-bound IgE on Langerhans cells facilitates binding of allergens to Langerhans cells prior to their processing and antigen presentation.

Autoallergens

IgE autoantibodies against human proteins have been demonstrated by Valenta et al. (61) in a high percentage of severe AD patients, suggesting a role for IgE autoreactivity in the pathogenesis of chronic AD. An increase in the serum level of IgE autoantibodies against the human protein *ara* (atopy-related IgE autoantigen) NAC (nascent polypeptide-associated complex) was shown to correlate with the severity of AD (62). Using the serum IgE from AD patients, a cDNA coding for an IgE autoantigen, *Hom s 1*, has recently been isolated and characterized (63). *Hom s 1* was found to be strongly expressed in human skin. In patients

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with these IgE autoantibodies, autoallergens could contribute to chronic AD by causing continuous activation of skin mast cells and arming FcεR1+ Langerhans cells for presentation of epidermal proteins to Th2 T cells in the skin. Since these are endogenous antigens that cannot be removed from the patient, the only recourse for therapy would be tolerization to the autoallergen or elimination of the patient's IgE.

Naturally Occurring Anti-IgE

Patients with AD also contain circulating autoantibodies to IgE, which can activate inflammatory cells bearing IgE (64). The functionality of these anti-IgE has been assessed by the ability of serum containing these autoantibodies to release histamine from basophils and mast cells (65). It was shown that anti-IgE from AD patients are 30 times more potent than rabbit anti-IgE for histamine release. In serum from AD patients as compared to those from normal controls, the IgG anti-IgE activity measured by the percentage of binding activity is significantly higher. Thus, the activation of IgE-bearing Langerhans cells, basophils, and macrophages by autoantibodies to IgE could contribute to the skin inflammation associated with AD.

Superantigenic Effects

Staphylococcal superantigens stimulate a polyclonal expansion of T lymphocytes expressing a specific population of T-cell receptor (TCR) variable chain V β genes and induce the release of high levels of cytokines from T cells and macrophages (reviewed in Ref. 66). Superantigens could exacerbate AD by several mechanisms. They could penetrate inflamed skin and engage HLA-DR on epidermal macrophages or Langerhans cells to stimulate the production of proinflammatory cytokines. They could also stimulate a high proportion of T cells expressing the CLA skin homing receptor to proliferate and secrete proinflammatory cytokines (67). Recently, we demonstrated that staphylococcal toxins at low concentrations are able to stimulate the in vitro production of allergen-specific IgE by B cells from AD patients (68). In addition, TSST-1 induced on B cells the expression of B7.2, a molecule recently demonstrated to enhance Th2 responses and to be involved in IgE regulation of B cells from AD patients (69). Thus, by acting on several arms of the immune response, superantigens can significantly augment allergic responses.

D. Anti-IgE in the Management of AD

Antigen-IgE complexes after binding with CD23 and possibly FcεRI have been shown to amplify the T-cell response to allergen by facilitating antigen presentation to antigen-specific T cells. This was illustrated in mice, where injection of

anti-IgE antibodies inhibited T-cell production of IL-4 and airway eosinophilia (70). In these experiments, the response was not mast cell dependent, but seemed to act directly through CD23 by interrupting the positive feedback loop of Th2 response and IgE. It has been suggested that nonanaphylactogenic anti-IgE antibodies may be useful in the therapy of allergic diseases. In humans with respiratory allergy, anti-IgE antibody administered by intravenous infusion was shown to be well tolerated and did not induce serum sickness or immune complex disease (71). A significant decrease in serum IgE was demonstrated in pollen-allergic patients with elevated IgE levels. Marked downregulation of FcεRI was also achieved after treatment with anti-IgE antibodies (72). Histamine release by basophils challenged by anti-IgE antibodies was marginally decreased after treatment, whereas histamine release after challenge with house dust mite allergen was reduced by 90%. In asthmatic patients, 12 weeks of therapy with anti-IgE antibodies resulted in improvement in both symptoms and pulmonary function tests. The treatment reduced IgE levels, FcεRI expression on basophils, and skin test reactivity to allergens. After discontinuation of treatment, FcεRI expression was restored and serum IgE increased again. In mice sensitized with ovalbumin, intravenous administration of anti-IgE inhibited antigen-induced skin reactions, even in mice where sensitivity was transferred passively by specific IgE injection (70). Since IgE plays a major role in the pathogenesis of AD by enhancing allergen presentation and allergen-induced skin inflammation, removal of IgE by use of anti-IgE antibody therapy may be worth pursuing.

III. Urticaria and Angioedema

A. Clinical Presentation

Urticaria is characterized by pruritic, erythematous circumscribed, raised areas of edema that are evanescent and involve the superficial portion of the dermis. When the edematous process extends into the deep dermis and subcutaneous tissue, it is known as angioedema (73). Urticaria may involve any part of the body, whereas angioedema is preferentially located on the face, the extremities, or the genitalia. Urticaria and angioedema may occur together or separately. Acute urticaria is self-limiting and frequently caused by an IgE-dependent allergic reaction to a food or a drug. Individuals with a positive family history of allergic diseases have an increased risk for developing IgE-mediated urticarial lesions. A comprehensive history and physical examination complemented by allergy skin prick testing for the suspected allergen(s) will help to find the etiology of acute urticaria.

Avoidance measures and oral double-blinded, placebo-controlled food challenges may be necessary to confirm the role of an allergen. Other substances may induce urticaria by non–IgE-mediated mechanisms, like additives, aspirin or

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nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, narcotics, and oral contraceptives. Infectious illnesses or psychological stress may also be implicated in the exacerbations of acute urticaria (74).

Urticaria is categorized as chronic if it occurs daily or almost daily for at least 6 weeks. It should not be confused with recurrent urticaria, which is due to repeated encountered allergens inducing a new flare on each occasion (74). In patients with chronic urticaria, a precise etiology is often not found despite tedious and laborious search for infections, neoplasia, food additive intolerance, or psychological disturbances. The term *chronic idiopathic urticaria* (CIU) is used for patients after exclusion of known causes of urticaria. In clinical practice, CIU generally represents more than 75% of patients seen, physical urticaria 15%, and other defined urticarias about 5% (74). In contrast to acute urticaria, the incidence of atopic manifestations, such as atopic dermatitis, allergic rhinitis, or asthma, is not increased in CIU, and their serum IgE levels are often within the normal limits. Usually the white blood count is normal and inflammatory markers are not increased. In its severe form, CIU may be extremely disabling and difficult to treat.

B. Pathogenesis

Acute Urticaria

This form of urticaria is usually due to an IgE-mediated reaction causing the release of biologically active factors from basophils and mast cells (Table 3). The initial response, occurring within minutes, is erythema caused by dilation of small blood vessels in the dermis. The wheal accompanying the erythema is due to increased capillary permeability causing transudation of fluid. Histamine is the major mediator involved in urticaria. Indeed, intradermal injection of histamine, as positive control for allergen skin prick tests, produces a wheal-and-flare response with pruritus similar to the urticarial lesions. Histamine is produced mainly by mast cells but also by basophils (73).

Cutaneous mast cells and their mediators play a central role in the pathogenesis of urticaria. In the skin, mast cells are predominantly of the type containing

Table 3 Evidence for IgE Involvement in a Subset of Urticaria

Higher prevalence of acute urticaria in atopic patients
Presence of allergen-specific IgE in patients with acute urticaria
Correlation between allergen exposure and flares of acute urticaria
Urticaria induced by cross-linking of FcεR1
Induction of wheal-and-flare reaction similar to acute urticaria by intradermal injection of an allergen
Presence of anti-IgE in a small subset of chronic idiopathic urticaria

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chymase and tryptase and are located close to blood vessels and sensory nerves (74,75). When activated, mast cells release secretory granules containing different mediators, in particular histamine. Mast cells also secrete prostaglandin D_2 and leukotriene C_4, which are vasoactive molecules and can cause urticaria-like reactions when injected in the skin (76). Cytokines, such as IL-4, IL-8, and TNF-α, are produced by mast cells contributing to neutrophil and eosinophil infiltration and lymphocyte activation (77–79). Mast cell activation and degranulation may be triggered by many immunological factors, such as allergens, anti-IgE, and anti-IgE-receptor autoantibodies or anaphylatoxins, and nonimmunological factors, like neuropeptides, hormones, medications, or physical stimuli. The localization of mast cells close to sensory nerves facilitates the effect of neuropeptides (vasoactive intestinal peptide, somatostatin, substance P) on mast degranulation (75).

Cutaneous mast cells stimulated by antigen binding to specific surface-bound IgE antibodies will result in a wheal-and-flare reaction and occasionally a late phase reaction, which peaks at 6–8 hours and resolves within 24–48 hours (80). In the late-phase reaction, there is not only vasodilation, but also development of a perivascular infiltrate consisting of neutrophils, eosinophils, and monocytes. The predominance of neutrophils may be observed in the first 24 hours and is replaced by a CD4+ T-cell reaction at 48 hours (81). The prominent role of IgE in acute urticaria is suggested by the presence of IgE to the allergen (food, drug, venom) involved in the serum of the patient and by the fact that transfer of IgE antibodies to a normal recipient followed by an antigen challenge will result in an urticarial reaction. Finally, in a sensitized individual, intradermal injection of an allergen will induce a wheal-and-flare reaction similar to urticaria (73).

Chronic Idiopathic Urticaria

Histological lesions of CIU are different from those of physical urticaria. In cold urticaria or dermographism, there is increased vascular permeability occurring within 2 hours but no late phase component, nor is there a cellular infiltrate. In CIU, the typical lesions consist of a perivascular mononuclear infiltrate but is not a necrotizing vasculitis. In some patients, a skin biopsy may reveal vasculitis with fibrinoid necrosis and a predominant neutrophilic infiltrate. These histological differences are not visible clinically, since vasculitic urticarial lesions can be clinically indistinguishable from those seen in the nonvasculitic urticaria (74). Immune complexes may play a role in the pathogenesis of chronic urticaria, since circulating immune complexes are found in 33% of the patients with vasculitis (82–84). The deposition of immune complexes can also be seen in skin biopsies, mainly in those with neutrophilic infiltrate. In skin biopsies of patients with CIU compared with normal controls, there is a 10-fold increase in mast cells and a fourfold increase in mononuclear cells. A predominance of helper T cells has

been demonstrated in the mononuclear cell infiltrate (85). Only a few patients have an eosinophilic infiltrate, but up to 40% show deposition of eosinophil major basic protein (86).

In the wheals of CIU, histamine concentrations are increased but the number of mast cells is not increased (85). This observation could be explained by an infiltration of basophils, which could play a role in the release of histamine during late phase reactions and be responsible for the maintenance of wheals in CIU. Basophils are similar to mast cells but do not produce prostaglandin D_2 and proteases. Unlike mast cells, basophils mature in the bone marrow and their activation is dependent on the presence of IL-3. Mast cells and basophils express FcεRI, the cross-linking of which induces degranulation (74).

Based on these observations, a mechanism involving cross-linking of the FcεRI has been postulated in CIU. Several studies have shown that intradermal injection of autologous serum induces an urticarial reaction locally, suggesting that histamine-releasing factors are present in the blood of patients with CIU (87). An autoimmune origin for CIU has been suspected because of the association of this disease with thyroid autoimmunity (88,89). Elevated titers of thyroid microsomal antibodies have been described in 12% of patients with urticaria, and about one half of these patients had a goiter or thyroid dysfunction. These antibodies are detected in only about 5% of control subjects. The association of thyroid autoantibodies in patients with chronic urticaria has been confirmed by multiple studies, but the mechanism for this association remains poorly understood (89). It has been suggested that these autoantibodies are not pathogenic but only markers for autoimmunity. A more direct link to autoimmunity was demonstrated when an association of anti-IgE and/or anti-IgE receptor autoantibodies was found in CIU.

Autoantibodies and Chronic Urticaria

In classical allergic reactions, FcεRI+ cells such as mast cells are triggered by cross-linking between the Fab portions of IgE and allergens. These cells may also be stimulated by three other mechanisms without involving antigens: anti-IgE antibodies (directed against the Fcε portion of IgE), antibodies against FcεRI, and anti-IgG acting on IgG-IgE complexes bound to FcεRI. Anti-IgE and anti-FcεRI autoantibodies have been found in chronic urticaria patients. A role for these antibodies in the pathogenesis of chronic urticaria has been postulated because of their ability to induce histamine release from mast cells and basophils located in the dermis of these patients. In one study, the presence of anti-IgE antibodies was assessed in the serum of patients with cold urticaria, urticarial vasculitis, and chronic urticaria and compared to normal controls (90). Anti-IgE antibodies were found in about the half of the patients in the three groups of urticaria, but not in the control group. The functional relevance of these antibodies

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was confirmed by the induction of basophil degranulation in vitro and a wheal-and-flare reaction after in vivo intradermal injection with the positive sera. One serum containing IgG anti-IgE from a patient with cold urticaria gave a positive transfer test, suggesting a pathogenic role for these antibodies (91).

Antibodies directed against FcεRI were recently described in chronic urticaria. In 32% of 37 patients with chronic urticaria, autoantibodies directed to FcεRI were found. The specificity of these antibodies to FcεRI was confirmed by their immunoprecipitation with FcεRIα from FcεRIαγ-transfected cells. These autoantibodies were not found in the serum from AD patients or healthy controls. In contrast, IgG anti-IgE were detected in the majority of the serum of chronic urticaria and AD patients and in some sera from healthy controls (91). Both types of antibodies proved to be functional by histamine release activity, but not in all patients (92). The autoantibodies against FcεRI in CIU occur mainly in the IgG1 and IgG3 subclasses (93).

In order to distinguish between functional autoantibodies against FcεRI and against IgE, histamine release assays have been used. In these experiments, basophils are obtained either from a healthy donor treated with lactic acid to remove cell-bound IgE or untreated basophils from a second donor who is atopic. In this assay, if the serum induces histamine release only from basophils of the first donor, it indicates the presence exclusively of anti-FcεRI autoantibodies. Stimulation of basophils of the second donor indicates the presence of anti-FcεRI or anti-IgE autoantibodies or both, and substantially more release from donor's two basophils demonstrates the predominant presence of anti-IgE autoantibodies (94). The incidence of anti-FcεRI autoantibodies was studied in 163 patients with CIU. Ninety-eight of these patients (60%) showed signs of mast cell degranulation after intradermal injection of autologous serum (95). Histamine release from basophils in vitro was found in 31% of the patients: 47 patients in the skin-test positive group and 3 in the skin-test negative group. In contrast, none of the sera of healthy donors induced histamine release from basophils. The sera releasing histamine from basophils also induced histamine from mast cells in incubated skin slices. The histamine release was IgG-mediated, and preincubation with recombinant α-chain from FcεRI inhibited histamine release from basophils and mast cells, supporting the concept that anti-FcεRI autoantibodies are relevant to the pathogenesis of chronic urticaria in about one fourth of the patients (95).

Another report found that 60% of the patients with chronic urticaria had functional antibodies directed to FcεRI and 10% anti-IgE autoantibodies. In these studies, the presence of anti-FcεRI and anti-IgE autoantibodies was correlated with the clinical features of 107 CIU patients (96). Patients with autoantibodies demonstrated significantly higher wheal scores, a higher number of involved sites, and association with higher itch scores. Associated systemic symptoms were significantly higher in patients with autoantibodies. Patients with autoantibodies seem to have more severe urticaria associated with low serum IgE and

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decreased basophil releasability and number. The type of cell infiltrate in skin urticarial lesions was also studied in patients with autoantibodies and compared to patients without autoantibodies (97). The only difference found was eosinophil activation, which occurred later and was more persistent in patients without autoantibodies. These data suggest that despite different triggers, mediator release from mast cells is similar in urticaria patients with or without autoantibodies. Overall these studies demonstrate that in a subset of patients chronic urticaria is an autoimmune disease, where autoantibodies directed against IgE or FcεRI play a role in the pathogenesis of the disease.

C. Anti-IgE and Therapy of Urticaria

In patients with IgE-dependent urticaria who cannot avoid allergens that trigger their symptoms, anti-IgE therapy may represent one approach to reduce allergen-induced mast cell degranulation. For CIU, removal of anti-IgE autoantibodies from the circulation may also be a therapeutic approach for those patients whose disease is triggered by anti-IgE autoantibodies. This approach, however, would probably not work in most patients with CIU who have antibodies to the high-affinity IgE receptor. In such patients, reduction of serum IgG by plasma exchange in skin test–positive CIU patients can improve their clinical state (98). The use of soluble recombinant-FcεRIα may be a promising therapeutic approach of CIU to neutralize anti-FcεRI autoantibodies. However, information about the immunogenicity of systemic use of soluble recombinant-FcεRIα is still lacking. A more effective form of treatment, however, may be tolerization of the B- and T-cell clones reactive to FcεRIα.

IV. Other Autoimmune Diseases with Skin Involvement

The potential pathogenic role of mast cells in several systemic autoimmune diseases has triggered a search for anti-IgE and anti-FcεRI autoantibodies detection in the serum of these patients (99). In this regard, IgM and IgG anti-IgE autoantibodies have been detected in the serum of patients with systemic lupus erythematosus and systemic sclerosis (100). The exact biological relevance of these autoantibodies is not known. In systemic lupus erythematosus, organ damage is secondary to the deposition of immune complexes within the small blood vessels. These complexes may contain IgE/IgG anti-IgE immune complexes. Furthermore, patients with active disease appear basopenic. This may reflect previous degranulation of basophils (101). In systemic sclerosis, it has been recently suspected that mast cells and their metabolites may play a role in the pathogenesis. However, sera of patients with anti-IgE autoantibodies were not able to degranulate basophils in vitro (99). Anti-FcεRI autoantibodies have also been detected in systemic lupus erythematosus (20%), dermatomyositis (36%), pemphigus vul-

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garis (39%), and bullous pemphigoid (13%) (93). In contrast to CIU, these autoantibodies do not release histamine from basophils and belong to different subclasses, IgG2 and IgG4. These observations underscore the fact that autoantibodies against IgE are heterogeneous immunochemically and functionally, explaining the different histamine-releasing activities of these antibodies. Different affinities for IgE, epitope specificity, and subclass composition may be an explanation for these heterogeneous reactions, but further investigations are needed to understand the role of these autoantibodies in the pathogenesis of autoimmune skin diseases.

V. Conclusions

IgE has a multifunctional role in the pathogenesis of allergic and autoimmune skin diseases. In both AD and acute urticaria, IgE plays a key role in the pathogenesis as illustrated by clinical and experimental data. Based on avoidance measures and provocation tests, it has been shown that IgE-mediated immune responses to allergens promote skin inflammation in both diseases. In AD, allergen-induced skin inflammation is mediated by IgE, the synthesis of which is enhanced by a Th2 cytokine environment. Furthermore, IgE binding to FcεRI on the surface of Langerhans cells has been shown to facilitate allergen presentation, which augments allergic skin inflammation. In acute urticaria and angioedema, allergens bind to IgE on the FcεRI of mast cells and basophils, thus inducing mediator release and subsequently an inflammatory response. However, in a majority of patients with CIU and anti-FcεRI, autoantibodies were suggested to be involved in the pathogenesis of skin inflammation. Based on the clinical and laboratory evidence that IgE plays a central role in the pathogenesis of AD and acute urticaria, the IgE molecule is likely to be a good target for treatment of these diseases. The use of anti-IgE treatment has not yet been studied in allergic skin diseases, but it may be worth pursuing.

References

1. Leung DYM, Tharp M, Boguniewicz M. Atopic dermatitis. In: Freeberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, Fitzpatrick TB, eds. Dermatology in General Medicine. New York: McGraw-Hill, 1998: 1464–1480.
2. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Dermato-Venereol 1980; 92:44–47.
3. Williams HC, Strachan DP. The natural history of childhood eczema: observations from the British 1958 birth cohort study. Br J Dermatol 1998; 139(5):834–839.
4. Schultz Larsen F. Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample. J Am Acad Dermatol 1993; 28(5 Pt 1):719–723.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

5. Williams H, Robertson C, Stewart A, Ait-Khaled N, Anabwani G, Anderson R, Asher I, Beasley R, Bjorksten B, Burr M, Clayton T, Crane J, Ellwood P, Keil U, Lai C, Mallol J, Martinez F, Mitchell E, Montefort S, Pearce N, Shah J, Sibbald B, Strachan D, von Mutius E, Weiland SK. Worldwide variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood. J Allergy Clin Immunol 1999; 103(1 Pt 1):125–138.
6. Buckley RH, Fiscus SA. Serum IgD and IgE concentrations in immunodeficiency diseases. J Clin Invest 1975; 55(1):157–165.
7. Saurat JH. Eczema in primary immune-deficiencies. Clues to the pathogenesis of atopic dermatitis with special reference to the Wiskott-Aldrich syndrome. Acta Derm Venereol Suppl 1985; 114:125–128.
8. Agosti JM, Sprenger JD, Lum LG, Witherspoon RP, Fisher LD, Storb R, Henderson WR, Jr. Transfer of allergen-specific IgE-mediated hypersensitivity with allogeneic bone marrow transplantation. N Engl J Med 1988; 319(25):1623–1628.
9. Borish L, Rosenwasser LJ. Update on cytokines. J Allergy Clin Immunol 1996; 97(3):719–733.
10. Renz H, Jujo K, Bradley KL, Domenico J, Gelfand EW, Leung DYM. Enhanced IL-4 production and IL-4 receptor expression in atopic dermatitis and their modulation by interferon-gamma. J Invest Dermatol 1992;99(4):403–408.
11. Akdis M, Akdis CA, Weigl L, Disch R, Blaser K. Skin-homing, CLA+ memory T cells are activated in atopic dermatitis and regulate IgE by an IL-13-dominated cytokine pattern: IgG4 counter-regulation by CLA- memory T cells. J Immunol 1997; 159(9):4611–4619.
12. Reinhold U, Pawelec G, Wehrmann W, Herold M, Wernet P, Kreysel HW. Immunoglobulin E and immunoglobulin G subclass distribution in vivo and relationship to in vitro generation of interferon-gamma and neopterin in patients with severe atopic dermatitis. Int Arch Allergy Appl Immunol 1988;87(2):120–126.
13. Jujo K, Renz H, Abe J, Gelfand EW, Leung DYM. Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. J Allergy Clin Immunol 1992; 90(3 Pt 1):323–331.
14. Wierenga EA, Snoek M, de Groot C, Chretien I, Bos JD, Jansen HM, Kapsenberg ML. Evidence for compartmentalization of functional subsets of CD2+ T lymphocytes in atopic patients. J Immunol 1990; 144(12):4651–4656.
15. Santamaria Babi LF, Picker LJ, Perez Soler MT, Drzimalla K, Flohr P, Blaser K, Hauser C. Circulating allergen-reactive T cells from patients with atopic dermatitis and allergic contact dermatitis express the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen. J Exp Med 1995; 181(5):1935–1940.
16. Mihm MC, Jr., Soter NA, Dvorak HF, Austen KF. The structure of normal skin and the morphology of atopic eczema. J Invest Dermatol 1976; 67(3):305–312.
17. Leung DYM, Bhan AK, Schneeberger EE, Geha RS. Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. J Allergy Clin Immunol 1983; 71(1 Pt 1):47–56.
18. Bos JD, Hagenaars C, Das PK, Krieg SR, Voorn WJ, Kapsenberg ML. Predominance of ''memory'' T cells (CD4+, CDw29+) over ''naive'' T cells (CD4+, CD45R+) in both normal and diseased human skin. Arch Dermatol Res 1989; 281(1):24–30.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

19. Leiferman KM, Ackerman SJ, Sampson HA, Haugen HS, Venencie PY, Gleich GJ. Dermal deposition of eosinophil-granule major basic protein in atopic dermatitis. Comparison with onchocerciasis. N Engl J Med 1985; 313(5):282–285.
20. Kagi MK, Joller-Jemelka H, Wuthrich B. Correlation of eosinophils, eosinophil cationic protein and soluble interleukin-2 receptor with the clinical activity of atopic dermatitis. Dermatology 1992; 185(2):88–92.
21. Hamid Q, Boguniewicz M, Leung DYM. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. J Clin Invest 1994; 94(2):870–876.
22. Hamid Q, Naseer T, Minshall EM, Song YL, Boguniewicz M, Leung DYM. In vivo expression of IL-12 and IL-13 in atopic dermatitis. J Allergy Clin Immunol 1996; 98(1):225–231.
23. Laberge S, Ghaffar O, Boguniewicz M, Center DM, Leung DYM, Hamid Q. Association of increased CD4+ T-cell infiltration with increased IL-16 gene expression in atopic dermatitis. J Allergy Clin Immunol 1998; 102(4 Pt 1):645–650.
24. Bratton DL, Hamid Q, Boguniewicz M, Doherty DE, Kailey JM, Leung DYM. Granulocyte macrophage colony-stimulating factor contributes to enhanced monocyte survival in chronic atopic dermatitis. J Clin Invest 1995; 95(1):211–218.
25. Grewe M, Bruijnzeel-Koomen CA, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, Krutmann J. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol Today 1998; 19(8):359–361.
26. Taha RA, Leung DYM, Ghaffar O, Boguniewicz M, Hamid Q. In vivo expression of cytokine receptor mRNA in atopic dermatitis. J Allergy Clin Immunol 1998; 102(2):245–250.
27. Taha RA, Leung DYM, Ghaffar O, Boguniewicz M, Hamid Q. In vivo expression of cytokine receptor mRNA in atopic dermatitis. J Allergy Clin Immunol 1998; 102(2):245–250.
28. Fujisawa T, Fukuda S, Atsuta J, Ichimi R, Kamiya H, Sakurai M. Interferon-gamma induces interleukin-3 release from peripheral blood eosinophils. Int Arch Allergy Immunol 1994; 104(suppl 1(1)):41–43.
29. Wittig HJ, Belloit J, De Fillippi I, Royal G. Age-related serum immunoglobulin E levels in healthy subjects and in patients with allergic disease. J Allergy Clin Immunol 1980; 66(4):305–313.
30. Leung DYM. Atopic dermatitis: the skin as a window into the pathogenesis of chronic allergic diseases. J Allergy Clin Immunol 1995; 96(3):302–318.
31. Hoffman DR, Yamamoto FY, Geller B, Haddad Z. Specific IgE antibodies in atopic eczema. J Allergy Clin Immunol 1975; 55:256–267.
32. Sampson HA, Albergo R. Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. J Allergy Clin Immunol 1984; 74(1):26–33.
33. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. N Engl J Med 1990; 323(8):502–507.
34. Holt PG. Immunoprophylaxis of atopy: light at the end of the tunnel? Immunol Today 1994; 15(10):484–489.
35. Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders. J Allergy Clin Immunol 1999; 103(5 Pt 1):717–728.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

36. Sampson HA. Food allergy. Part 2: diagnosis and management. J Allergy Clin Immunol 1999; 103(6):981–989.
37. Sampson HA, Jolie PL. Increased plasma histamine concentrations after food challenges in children with atopic dermatitis. N Engl J Med 1984; 311(6):372–376.
38. Sampson HA, Broadbent KR, Bernhisel-Broadbent J. Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. N Engl J Med 1989; 321(4):228–232.
39. Guillet G, Guillet MH. Natural history of sensitizations in atopic dermatitis. A 3-year follow-up in 250 children: food allergy and high risk of respiratory symptoms. Arch Dermatol 1992; 128(2):187–192.
40. Mitchell EB, Crow J, Chapman MD, Jouhal SS, Pope FM, Platts-Mills TA. Basophils in allergen-induced patch test sites in atopic dermatitis. Lancet 1982; 1(8264): 127–130.
41. Clark RA, Adinoff AD. The relationship between positive aeroallergen patch test reactions and aeroallergen exacerbations of atopic dermatitis. Clin Immunol Immunopathol 1989; 53(2 Pt 2):S132–140.
42. Tan BB, Weald D, Strickland I, Friedmann PS. Double-blind controlled trial of effect of housedust-mite allergen avoidance on atopic dermatitis. Lancet 1996; 347(8993):15–18.
43. Kieffer M, Bergbrant IM, Faergemann J, Jemec GB, Ottevanger V, Stahl Skov P, Svejgaard E. Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrheic dermatitis. J Am Acad Dermatol 1990; 22(5 Pt 1):739–742.
44. Rokugo M, Tagami H, Usuba Y, Tomita Y. Contact sensitivity to *Pityrosporum ovale* in patients with atopic dermatitis. Arch Dermatol 1990; 126(5):627–632.
45. Back O, Scheynius A, Johansson SG. Ketoconazole in atopic dermatitis: therapeutic response is correlated with decrease in serum IgE. Arch Dermatol Res 1995; 287(5): 448–451.
46. Kolmer HL, Taketomi EA, Hazen KC, Hughs E, Wilson BB, Platts-Mills TA. Effect of combined antibacterial and antifungal treatment in severe atopic dermatitis. J Allergy Clin Immunol 1996; 98(3):702–707.
47. Leyden JJ, Marples RR, Kligman AM. *Staphylococcus aureus* in the lesions of atopic dermatitis. Br J Dermatol 1974; 90(5):525–530.
48. Hauser C, Wuethrich B, Matter L, Wilhelm JA, Sonnabend W, Schopfer K. *Staphylococcus aureus* skin colonization in atopic dermatitis patients. Dermatologica 1985; 170(1):35–39.
49. Lever R, Hadley K, Downey D, Mackie R. Staphylococcal colonization in atopic dermatitis and the effect of topical mupirocin therapy. Br J Dermatol 1988; 119(2): 189–198.
50. Leung DYM, Harbeck R, Bina P, Reiser RF, Yang E, Norris DA, Hanifin JM, Sampson HA. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. J Clin Invest 1993; 92(3):1374–1380.
51. Nissen D, Pedersen LJ, Skov PS, Vejlsgaard GL, Poulsen LK, Jarlov JO, Karlsmark T, Nolte H. IgE-binding components of staphylococcal enterotoxins in patients with atopic dermatitis. Ann Allergy Asthma Immunol 1997; 79(5):403–408.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

52. Bunikowski R, Mielke M, Skarabis H, Herz U, Bergmann RL, Wahn U, Renz H. Prevalence and role of serum IgE antibodies to the *Staphylococcus aureus*-derived superantigens SEA and SEB in children with atopic dermatitis. J Allergy Clin Immunol 1999; 103(1 Pt 1):119–124.
53. Herz U, Schnoy N, Borelli S, Weigl L, Kasbohrer U, Daser A, Wahn U, Kottgen E, Renz H. A human-SCID mouse model for allergic immune response bacterial superantigen enhances skin inflammation and suppresses IgE production. J Invest Dermatol 1998; 110(3):224–231.
54. Kay AB, Ying S, Varney V, Gaga M, Durham SR, Moqbel R, Wardlaw AJ, Hamid Q. Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. J Exp Med 1991; 173(3): 775–778.
55. Leung DYM, Schneeberger EE, Siraganian RP, Geha RS, Bhan AK. The presence of IgE on macrophages and dendritic cells infiltrating into the skin lesion of atopic dermatitis. Clin Immunol Immunopathol 1987; 42(3):328–337.
56. Bruijnzeel-Koomen CA, Mudde GC, Bruijnzeel PL. The presence of IgE molecules on epidermal Langerhans cells in atopic dermatitis and their significance for its pathogenesis. Allerg Immunol (Paris) 1989; 21(6):219–223.
57. Klubal R, Osterhoff B, Wang B, Kinet JP, Maurer D, Stingl G. The high-affinity receptor for IgE is the predominant IgE-binding structure in lesional skin of atopic dermatitis patients. J Invest Dermatol 1997; 108(3):336–342.
58. Vercelli D, Jabara HH, Lee BW, Woodland N, Geha RS, Leung DYM. Human recombinant interleukin 4 induces Fc epsilon R2/CD23 on normal human monocytes. J Exp Med 1988; 167(4):1406–1416.
59. Rouzer CA, Scott WA, Hamill AL, Liu FT, Katz DH, Cohn ZA. Secretion of leukotriene C and other arachidonic acid metabolites by macrophages challenged with immunoglobulin E immune complexes. J Exp Med 1982; 156(4):1077–1086.
60. Mudde GC, Van Reijsen FC, Boland GJ, de Gast GC, Bruijnzeel PL, Bruijnzeel-Koomen CA. Allergen presentation by epidermal Langerhans' cells from patients with atopic dermatitis is mediated by IgE. Immunology 1990; 69(3):335–341.
61. Valenta R, Maurer D, Steiner R, Seiberler S, Sperr WR, Valent P, Spitzauer S, Kapiotis S, Smolen J, Stingl G. Immunoglobulin E response to human proteins in atopic patients. J Invest Dermatol 1996; 107(2):203–208.
62. Natter S, Seiberler S, Hufnagl P, Binder BR, Hirschl AM, Ring J, Abeck D, Schmidt T, Valent P, Valenta R. Isolation of cDNA clones coding for IgE autoantigens with serum IgE from atopic dermatitis patients. Faseb J 1998; 12(14):1559–1569.
63. Valenta R, Natter S, Seiberler S, Wichlas S, Maurer D, Hess M, Pavelka M, Grote M, Ferreira F, Szepfalusi Z, Valent P, Stingl G. Molecular characterization of an autoallergen, Hom s 1, identified by serum IgE from atopic dermatitis patients. J Invest Dermatol 1998; 111(6):1178–1183.
64. Quinti I, Brozek C, Wood N, Geha RS, Leung DYM. Circulating IgG autoantibodies to IgE in atopic syndromes. J Allergy Clin Immunol 1986; 77(4):586–594.
65. Marone G, Casolaro V, Paganelli R, Quinti I. IgG anti-IgE from atopic dermatitis induces mediator release from basophils and mast cells. J Invest Dermatol 1989; 93(2):246–252.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

66. Kotzin BL, Leung DYM, Kappler J, Marrack P. Superantigens and their potential role in human disease. Adv Immunol 1993; 54:99–166.
67. Leung DYM, Gately M, Trumble A, Ferguson-Darnell B, Schlievert PM, Picker LJ. Bacterial superantigens induce T cell expression of the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen, via stimulation of interleukin 12 production. J Exp Med 1995; 181(2):747–753.
68. Hofer MF, Harbeck RJ, Schlievert PM, Leung DYM. Staphylococcal toxins augment specific IgE responses by atopic patients exposed to allergen. J Invest Dermatol 1999; 112(2):171–176.
69. Jirapongsananuruk O, Hofer MF, Trumble AE, Norris DA, Leung DYM. Enhanced expression of B7.2 (CD86) in patients with atopic dermatitis: a potential role in the modulation of IgE synthesis. J Immunol 1998; 160(9):4622–4627.
70. Heusser CH, Wagner K, Bews JP, Coyle A, Bertrand C, Einsle K, Kips J, Eum SY, Lefort J, Vargaftig BB. Demonstration of the therapeutic potential of non-anaphylactogenic anti-IgE antibodies in murine models of skin reaction, lung function and inflammation. Int Arch Allergy Immunol 1997; 113(1–3):231–235.
71. Corne J, Djukanovic R, Thomas L, Warner J, Botta L, Grandordy B, Gygax D, Heusser C, Patalano F, Richardson W, Kilchherr E, Staehelin T, Davis F, Gordon W, Sun L, Liou R, Wang G, Chang TW, Holgate S. The effect of intravenous administration of a chimeric anti-IgE antibody on serum IgE levels in atopic subjects: efficacy, safety, and pharmacokinetics. J Clin Invest 1997; 99(5):879–887.
72. MacGlashan DW, Jr., Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, Sterbinsky SA, Hamilton RG, Lichtenstein LM. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol 1997; 158(3):1438–1445.
73. Tharp MD. Chronic urticaria: pathophysiology and treatment approaches. J Allergy Clin Immunol 1996;98(6 Pt 3):S325–330.
74. Sabroe RA, Greaves MW. The pathogenesis of chronic idiopathic urticaria. Arch Dermatol 1997; 133(8):1003–1008.
75. Bienenstock J, Tomioka M, Matsuda H, Stead RH, Quinonez G, Simon GT, Coughlin MD, Denburg JA. The role of mast cells in inflammatory processes: evidence for nerve/mast cell interactions. Int Arch Allergy Appl Immunol 1987;82(3–4): 238–243.
76. Soter NA, Lewis RA, Corey EJ, Austen KF. Local effects of synthetic leukotrienes (LTC4, LTD4, LTE4, and LTB4) in human skin. J Invest Dermatol 1983; 80(2): 115–119.
77. Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, Bews JP, Hunt TC, Okayama Y, Heusser CH, et al. Interleukin 4 is localized to and released by human mast cells. J Exp Med 1992; 176(5):1381–1386.
78. Moller A, Lippert U, Lessmann D, Kolde G, Hamann K, Welker P, Schadendorf D, Rosenbach T, Luger T, Czarnetzki BM. Human mast cells produce IL-8. J Immunol 1993; 151(6):3261–3266.
79. Walsh LJ, Trinchieri G, Waldorf HA, Whitaker D, Murphy GF. Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocyte adhesion molecule 1. Proc Natl Acad Sci USA 1991; 88(10):4220–4224.

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80. Frew AJ, Kay AB. The pattern of human late-phase skin reactions to extracts of aeroallergens. J Allergy Clin Immunol 1988;81(6):1117–1121.
81. Solley GO, Gleich GJ, Jordon RE, Schroeter AL. The late phase of the immediate wheal and flare skin reaction. Its dependence upon IgE antibodies. J Clin Invest 1976; 58(2):408–420.
82. Monroe EW. Urticarial vasculitis: an updated review. J Am Acad Dermatol 1981; 5(1):88–95.
83. Monroe EW, Schulz CI, Maize JC, Jordon RE. Vasculitis in chronic urticaria: an immunopathologic study. J Invest Dermatol 1981; 76(2):103–107.
84. Mathison DA, Arroyave CM, Bhat KN, Hurewitz DS, Marnell DJ. Hypocomplementemia in chronic idiopathic urticaria. Ann Intern Med 1977; 86(5):534–538.
85. Kaplan AP, Horakova Z, Katz SI. Assessment of tissue fluid histamine levels in patients with urticaria. J Allergy Clin Immunol 1978; 61(6):350–354.
86. Grattan CE, Winkelmann RK, Leiferman KM. Eosinophil major basic protein in autologous serum and saline skin tests in chronic idiopathic urticaria [letter]. Br J Dermatol 1997; 136(1):141–142.
87. Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza Black A, Greaves MW. The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. Br J Dermatol 1999; 140(3):446–452.
88. Leznoff A, Josse RG, Denburg J, Dolovich J. Association of chronic urticaria and angioedema with thyroid autoimmunity. Arch Dermatol 1983; 119(8):636–640.
89. Heymann WR. Chronic urticaria and angioedema associated with thyroid autoimmunity: review and therapeutic implications. J Am Acad Dermatol 1999; 40(2 Pt 1):229–232.
90. Gruber BL, Baeza ML, Marchese MJ, Agnello V, Kaplan AP. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. J Invest Dermatol 1988; 90(2):213–217.
91. Fiebiger E, Maurer D, Holub H, Reininger B, Hartmann G, Woisetschlager M, Kinet JP, Stingl G. Serum IgG autoantibodies directed against the alpha chain of Fc epsilon RI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? J Clin Invest 1995; 96(6):2606–2612.
92. Tong LJ, Balakrishnan G, Kochan JP, Kinet JP, Kaplan AP. Assessment of autoimmunity in patients with chronic urticaria. J Allergy Clin Immunol 1997; 99(4): 461–465.
93. Fiebiger E, Hammerschmid F, Stingl G, Maurer D. Anti-Fc epsilon RI alpha autoantibodies in autoimmune-mediated disorders. Identification of a structure-function relationship. J Clin Invest 1998; 101(1):243–251.
94. Hide M, Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. N Engl J Med 1993; 328(22):1599–1604.
95. Niimi N, Francis DM, Kermani F, O'Donnell BF, Hide M, Kobza-Black A, Winkelmann RK, Greaves MW, Barr RM. Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. J Invest Dermatol 1996; 106(5):1001–1006.
96. Sabroe RA, Seed PT, Francis DM, Barr RM, Black AK, Greaves MW. Chronic idiopathic urticaria: comparison of the clinical features of patients with and without

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anti-Fc epsilon RI or anti-IgE autoantibodies. J Am Acad Dermatol 1999; 40(3): 443–450.
97. Sabroe RA, Poon E, Orchard GE, Lane D, Francis DM, Barr RM, Black MM, Black AK, Greaves MW. Cutaneous inflammatory cell infiltrate in chronic idiopathic urticaria: comparison of patients with and without anti-Fc epsilon RI or anti-IgE autoantibodies. J Allergy Clin Immunol 1999; 103(3 Pt 1):484–493.
98. Grattan CE, Francis DM, Slater NG, Barlow RJ, Greaves MW. Plasmapheresis for severe, unremitting, chronic urticaria. Lancet 1992; 339(8801):1078–1080.
99. Marone G, Spadaro G, Palumbo C, Condorelli G. The anti-IgE/anti-Fc epsilon RI alpha autoantibody network in allergic and autoimmune diseases. Clin Exp Allergy 1999; 29(1):17–27.
100. Gruber BL, Kaufman LD, Marchese MJ, Roth W, Kaplan AP. Anti-IgE autoantibodies in systemic lupus erythematosus. Prevalence and biologic activity. Arthritis Rheum 1988; 31(8):1000–1006.
101. Egido J, Sanchez Crespo M, Lahoz C, Garcia R, Lopez-Trascasa M, Hernando L. Evidence of an immediate hypersensitivity mechanism in systemic lupus erythematosus. Ann Rheum Dis 1980; 39(4):312–317.

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17

Role of IgE and Food Allergens

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I. Introduction

Allergy, including food allergy, is inflammatory processes in which preformed and newly synthesized mediators from mast cells and basophils, are released following crosslinking of specific IgE on their surface membranes that contribute to the symptoms and clinical condition. Allergic reactions to foods can range in severity from an itchy tongue in some individuals to life-threatening swelling of the throat, asthma, and anaphylaxis in others. Consequently, food allergy, as compared to adverse food reactions, represents a complex and heterogeneous immune deviation from the normal protective immune response in which the production of systemic antigen/allergen specific IgE plays an important role in the pathogenesis of food allergy. Much of the difficulty in discussing adverse food reactions and food allergy has been the nomenclature used in the literature. To resolve these issues, the American Academy of Allergy and Immunology issued guidelines to standardize the literature (Table 1) (1).

It is perfectly clear that food allergies, like other allergies, are inherited. However, other factors that are just as important in determining whether or not individuals will have food allergies include the time of life when first exposed to the food allergen, the amount of the food allergen exposed to at the time of

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Table 1 Definition of Terms

Adverse food reaction: a general term applied to clinically abnormal responses to an ingested food or food additive
Food allergy: (hypersensitivity) an immunological reaction resulting from ingestion of food or food additive
Food anaphylaxis: IgE-mediated classic allergic, systemic hypersensitivity
Food intolerance[a]: an abnormal physiological response to a food or food additive
Idiosyncratic: an anaphylactoid reaction that represents abnormal responses that differ from physiological or pharmacological effects; non–immune-mediated; e.g., response to radio contrast media
Metabolic: a metabolic effect of the host (intestinal insufficiency); e.g., carbohydrate (lactose) intolerance
Pharmacological: drug-like effect; e.g., caffeine in coffee and tyramine in cheeses
Food toxicity: a direct effect on the host without involvement of immune mechanisms
Toxins: either present in foods or released by microorganisms or parasites contaminating the food (bacterial = botulism; ''red tide'' = dinoflagellates eaten by mussels, clams, oysters; algal = ciguarta)

[a] Although the immune system is not responsible for the symptoms of food intolerance, the symptoms of food intolerance can resemble those of food allergy and must be differentiated accordingly.

sensitization, whether or not the gastrointestinal epithelial barrier can be penetrated by the food allergen, and the status of the immune system at the time of early exposure or subsequent exposure to the food allergen.

Sensitization can occur in utero as the developing fetus (as early as week 20) is nourished by the mother, and small, but immunologically intact incompletely digested food proteins can be transferred to the fetus in early embryonic life. Later in gestation, sensitization can take place via the amniotic fluid that the fetus swallows. If the fetus is genetically programmed to develop IgE at this time, sensitization and food allergy can result. Obviously, what the mother eats during pregnancy is important and can contribute to the future food allergic state of the developing fetus.

Sensitizations after birth can occur by either food allergens in the mother's breast milk or by foods that become part of the infant's diet. Newborns have an immature gastrointestinal epithelium that does not fully mature until the end of the first year of life; however, the immune system is fully capable of recognizing and processing the allergen to react vigorously when the allergen is encountered again. The response is the typical immediate hypersensitivity reaction.

The true prevalence of adverse food reactions is unknown. In the United States, there is a public perception of 20–30% that one member of a household

family has a food allergy (2). A consensus of the medical literature suggests that approximately 6–8% of young children and only 1% of adults have some type of food allergy (3).

Although extensive in vitro allergenic cross-reactivity in the legume family has been documented in some adults, a clinical study of 57 patients with legume sensitivity and in vitro cross-allergenicity with peanuts, soybeans, peas, and lima beans revealed that IgE cross-reactivity did not necessarily indicate clinical reactivity in young children (4). Examples of animal groups include birds (chicken, duck), crustaceans (crab, lobster), and red meats (beef, veal); plant groups include cereal (wheat, corn), legumes (peanut, lentil), fruits (apple, pear), and tree nuts (walnut, pecan). The cross-reactivity not only exists among these and other food groups, but also extends to nonfood allergens such as pollens, latex, and dust mites. A difference in clinical sensitivity and clinical reactivity among these food sources has achieved a much better understanding in recent years. Although it is an IgE immunological mechanism and a Th2 response is considered to be dominant in gastrointestinal food allergy, the basic mechanisms of food allergy are still not fully understood.

II. Characterization of Food Allergens

Foods are composed of proteins, carbohydrates, lipids, and food additives. The majority of food allergens usually represent the major fraction of the total protein content of the food source. Food allergens have been characterized as glycosylated proteins with acidic isoelectric points, with pI ranging from 4 to 6 and relatively small molecular weights, ranging from 10 to 60 kDa (5). Classification of food allergens is often based on solubility: water-soluble (albumins) and saline soluble (globulins) allergens. Although classified as such, comparisons of primary amino acid sequences of food allergens have not revealed common sequence motifs.

Food extracts to date have not been standardized by allergen content or biological activity. Allergens have been identified by both common name (e.g., milk allergens, β-lactoglobulin) and by genus-species-Arabic numeral (e.g., peanut allergens, *Arachis hypogaea*, Ara h 1). The generally accepted terminology designating either major or minor allergens depends on whether greater or less than 50% of the patients tested have corresponding allergen-specific IgE's (6). Isoallergens have been identified within food sources as proteins with similar molecular weight size, identical biological function, and $>50\%$ amino acid sequence identity.

Although there are no known unique biochemical or immunochemical characteristics of food allergens, they are generally stable to heat treatment, acids,

and proteases. Food allergens cross the intestinal mucosal barriers and represent immunogenic properties with a minimum of two IgE-binding sites. Many food sources are resistant to digestion, and treatment of crude extracts or food allergens with acid/protease concentrations simulating stomach conditions typically has little effect on the specific binding of allergen-specific IgE. There are exceptions to this rigid stability, such as the allergens identified in fresh fruits and some vegetables, which are extremely labile.

A protein that acts as an allergen is not recognized by the immune system as a discrete molecule. However, IgE specifically recognizes relatively small portions of the molecule (epitopes of 8–12 amino acids). Epitopes on protein molecules may be localized sequentially in the primary structure or arranged together due to secondary or tertiary structure linear and conformational epitopes, respectively. No common amino acid motif has been identified as an epitope. Carbohydrate structures on proteins may influence the allergenicity of the food. Some carbohydrate IgE antibodies have been identified in patient serum. IgE antibodies are able to recognize unrelated glycoproteins to specific allergens if the glycoprotein contains *N*-linked $\beta 1 \rightarrow 2$ xylose and $\alpha 1 \rightarrow 3$ fucose residues common and unique to plant glycoproteins. It has been suggested that the involvement of these xylose- and fucose-containing complex glycans in allergenic responses is underestimated. These glycans can provide a structural basis that may explain the cross-reactivities often observed between pollen, vegetable food, and insect allergen (7). Carbohydrates may also influence the secondary and tertiary structure of proteins and may therefore strongly determine conformational B-cell epitopes.

Exposure/sensitization levels are unknown; however, individuals with preexisting IgE-mediated food allergies can respond to minute levels of the offending allergen in a food source. Microgram to milligram quantities of peanuts can elicit adverse reactions in controlled food challenges in selected individuals. The specific immunochemical and physicochemical properties that account for the unique allergenicity in predisposed individuals are still poorly understood.

III. Pathophysiology of IgE-Mediated Food Allergy

The pathogenesis of food allergy involves three main areas: the food allergen, the gastrointestinal barrier, and the individual's genetic predisposition to the development of an allergic response. Many elements of the gastrointestinal (GI) tract physiology play important roles in the ultimate allergenicity of food proteins. These include the pH, digestive enzymes, peristalsis, transient time, resident bacteria and bacterial fermentation, and the intestinal barrier function, permeability, and absorption. It is generally accepted that the immaturity of the local immunity in the gastrointestinal tract or a breakdown of oral tolerance is involved in sensiti-

zation in infancy. However, little is known about its development in adults where impaired gastrointestinal immunity is unlikely. In the gastrointestinal tract uptake of food antigens is routed through specialized epithelial membranous (M) cells overlying the Peyer's patches, enterocytes and through intercellular gaps prior to processing and presentation to gut-associated lymphoid tissue. Under normal circumstances, food antigen exposure via the GI tract results in local IgA responses and preferential suppression of systemic IgM, IgG, and IgE antibody production as well as cell-mediated immune responses to food antigen. This physiological phenomenon is known as oral tolerance. In the genetically susceptible host, the lack of oral tolerance induction results in the development of food hypersensitivity responses (8,9). The appearance of adverse reactions in adults may result from sensitization by alternative routes, such as the respiratory tract, by which aeroallergens sharing homologous epitopes with some food allergens can induce the synthesis of specific IgE antibodies able to cross-react with different allergen sources evoking a clinical response.

Immediate hypersensitivity to foods, like other allergies, includes the interaction of food-specific IgE with Fcε receptors on basophils and mast cells and the subsequent release of mediators. Increased levels of plasma histamine have been associated with the development of urticaria, laryngeal edema, wheezing, vomiting, diarrhea, and hypotension following blinded food challenges. The interaction between surface-bound IgE on mast cells and basophils with food allergens has been evidenced by immediate wheal and erythema responses in the skin, by demonstration of mast cell degranulation in gastrointestinal biopsies, or by elevated histamine levels (10). Interestingly, both a failure to identify serum tryptase (11) and elevated levels of serum tryptase (12), a marker of mast cell degranulation, have been detected in some patients with near-fatal or fatal food-induced anaphylaxis. However, basophils from food-sensitive children were found to have high rates of spontaneous histamine release and to release histamine after challenge with specific food antigen (13). Although increased spontaneous histamine release has been associated with increased secretion of a histamine-releasing factor (HRF), suggestions to measure HRF in patients with food allergy have not been supported (13).

The duality of Th1 and Th2 T-helper cells that exists in murine animals probably also exists in humans (14). It has been postulated that IL-4 enhancement promotes the development of IgE-mediated hypersensitivity disorders such as food allergy, while the combination of defective INF-γ with enhanced IL-4 production promotes inflammatory atopic disorders such as atopic dermatitis and asthma. IgE immunoregulation suggests that the magnitude of the IgE response to persistent allergens depend upon this balance between T-helper cells and their cytokine production. However, environmental and other as yet unidentified factors also contribute to the allergic state of the predisposed individual.

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IV. Major Foods/Allergens Involved with IgE-Mediated Disease

Peanuts, tree nuts, fish, and shellfish account for the majority of food hypersensitivity reactions in adults. Milk, eggs, and peanuts cause >80% of food hypersensitivity reactions in children. Worldwide, there are differences in which foods cause hypersensitivity reactions in both children and adults that are primarily attributed to the diet of the existing population (15). Levels of 6 kU of allergen-specific IgE/L for egg, 32 kU of allergen-specific IgE/L for milk, 15 kU of allergen-specific IgE/L for peanut, and 20 kU allergen-specific IgE/L for fish were determined to be predictive of clinical reactivity with greater than 95% certainty (16). Predictive levels of allergen-specific IgE for soy and wheat could not be determined due to extensive cross-reactivity with homologous proteins from other food sources.

With the increased awareness of cross-reactivity of allergens from botanical and animal sources identified by in vitro techniques, including skin prick test, RAST/ELISA, and IgE-immunoblot analysis, the definition of major allergens has increased dramatically in the last few years. It may be time for investigators to be more critical in establishing the identity of major allergens. A definition of major allergens might be better based on the proteins causing a reaction in 80% of subjects with high levels of specific IgE antibodies and clinical symptoms demonstrated by challenge tests with validated methods.

A. Cow's Milk

Hippocrates (469–370 B.C.) was the first person to report that cow's milk could cause upset stomach and hives. He recognized that only certain men reacted adversely to cheese and suggested that these men produced an unusual body substance (''humour'') that caused them to react: ''Cheese does not harm all men alike; some can eat their fill of it without the slightest hurt, nay, those it agrees with are wonderfully strengthened thereby. Others come off badly. So the constituents of these men differ, and the difference lies in the constituent of the body which is hostile to cheese, and is roused and stirred to action under its influence. Those in whom a humour of such kind is present in greater quantity and with greater control over the body naturally suffer more severely. But if cheese were bad for the human constitution without exception it would hurt all.''

The prevalence of cow's milk allergy in infants and children worldwide is estimated to be 2.0–2.5% (17). Appearance of allergic symptoms related to cow's milk allergy often begins early in childhood but is generally lost in the first 3–5 years of life (18,19). Cow's milk consists of different proteins, caseins and whey proteins, representing 80% and 20% of total protein, respectively (20). Caseins were originally defined as phosphoproteins that precipitated from raw

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skim milk at a pH of 4.6 at 20°C; whey proteins were those proteins that remained in solution. Most patients who are allergic to cow's milk proteins have IgE antibodies to one or more of these classified proteins. The nomenclature of milk proteins utilizes the Greek letter with or without a subscript preceding the class name; the genetic variation is indicated by an uppercase Arabic letter with or without a numerical superscript following the class name.

Foods containing small quantities of milk, ranging from 0.04 to 1.1%, have been shown to produce allergy (21). Sensitivity to milk protein constituents has been reported for β-lactoglobulin, 82%; caseins, 43%; α-lactoglobulin, 41%; and serum albumin, 18% (22). (Table 2). Bovine β-lactoglobulin allergenicity has been considered to be allergenic because of its absence in human milk. Casein corresponds to an association of four different proteins (αS1-, β-, αS2-, and κ-casein), combined with the genetic variants, represent a wide number of potential allergenic epitopes. αS1-caseins (~32 kDa) and β-lactoglobulin (~27 kDa) appear to be the major allergens (23). Spuergin et al., using 118 peptides, 10 residues offset by 1 residue, identified IgE- and IgG-binding epitopes of α-S1-casein to essentially the same regions (amino acids 19–30, 93–98, 141–150) distinguished by a high content of nonpolar and aromatic amino acids (24). Nakajima-Adachi et al., using 13 peptides, 20 residues offset by 5 residues, in combination with digestion fragments, identified a 20-amino-acid peptide designated P13 at the carboxyl terminus (25). These two studies reported slightly different results. However, different structural analysis of the protein, different formulas used to determine the level of IgE binding, and different allergic patient populations (Germany vs. Japan) might account for the differences identified, respectively. Using T-cell lines from two individuals in their study, Nakajima-Adachi et al. were able to identify different amino acid motif (25). The motifs represented were (-E-$(X)_7$-L-) and (-E-$(X)_6$-K-), which is similar to a cross-reacting T-cell epitope on a number of inhalant allergens (-E-$(X)_5$-K-). The relatedness of these and other similar epitopes requires further investigation before any significance can be attributed to this motif.

Additional whey proteins, including β-lactoglobulin and bovine serum albumins, are also allergenic. Bovine serum albumin (67 kDa) comprises approximately 1% of total milk protein and is quite heterogeneous in nature, with several different isoforms.

B. Eggs

Chicken (*Gallus domesticus*) eggs have been used as a nutrient source throughout human history. Egg is an inherent ingredient in human and animal food and is most difficult to exclude from the diet. Several studies have documented the major allergens in eggs (26–28). Egg white (albumin) is more allergenic than egg yolk. Egg yolk, which can be separated into two fractions by ultracentrifugation,

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Table 2 Common Food Allergens

Food		Molecular weight (daltons)	Comments
Milk			
Caseins		19,000–24,000	
Alpha		23,600–25,200	
Beta		24,000	
Kappa		19,000	
Gamma		18,300	
Whey			
β-Lactoglobulin		18,300	
α-Lactalbumin		14,100	
Bovine serum albumin		67,000	
Peanut			
Ara h 1		63,500	7S (Vicilin) seed storage protein, reduced form
Ara h 2		17,000	Conglutin, seed storage protein, reduced form
Ara h 3		60,000	Glycinin seed storage protein, reduced form
Peanut agglutinin		120,000	Minor allergen
Soybean			
SBTI		20,500	Seed protein
Gly m 1		7,500	Seed hull aeroallergen
Gly m 2		7,000	Seed hull aeroallergen
Gly m Bd30K		34,000	Protein storage vacuole protein
Chicken egg			
Egg white proteins			
Ovalbumin	Gal d 1	44,500	
Ovomucoid	Gal d 2	28,000	
Ovotransferrin	Gal d 3	76,000	
Lysozyme	Gal d 4	16,200	
Egg yolk proteins			
Lipovitellin		400,000	
Livitin		45,000–150,000	
Shrimp			
Tropomyosin		34,000–36,000	Heat-stable allergens
Met e 1		36,000	Greasyback shrimp
Pen a 1		34,000	Brown shrimp
Pen I 1		36,000	Indian shrimp
Mustard			
Sin a 1		14,200	2S Albumin superfamily, yellow mustard
Bra j 1		14,600	2S Albumin superfamily, Oriental mustard
Rice			
Ory s 1		14,800	Cereal trypsin/α-amylase family
Apple			
Mal d 1		17,500	Bet v 1 pathogenesis-related protein family
Barley			
Hor v 1		14,500	Cereal trypsin/α-amylase family
Brazil nut			
Ber e 1		12,000	2S Albumin superfamily
Celery			
Api g 1		16,300	Bet v 1 pathogenesis-related protein family
Codfish			
Gad c 1		12,000	Parvalbumin, allergen M

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contains a granular fraction consisting of lipovitellin, phosvitin, and low-density lipoproteins and a supernatant fraction that contains primarily lipid.

Five major components, ovalbumin (OA), ovomucoid (OM), ovotransferrin (Otr), ovomucin (Omc), and lysozyme (Lyz), account for 80% of the total egg protein content. The major allergen (Gal d 1) is an ovomucoid acidic (pI 4) glycoprotein representing ~10% of total egg protein, with a molecular weight of 28 kDa (28). Ovomucoid has been shown to have significant homology to pancreatic trypsin inhibitor. The intact protein is arranged into the three domains secured by interdomain disulfide bridges. In vitro binding studies performed with IgE and IgG epitopes show the allergen to be stable to extensive heating, proteolytic digestion, deglycosylation, and chemical denaturation (29).

Ovalbumin (Gal d 2) is a monomeric phosphoglycoprotein representing >50% of total egg white protein, with three primary variants, A_1, A_2, A_3, having an apparent molecular weight of 40–45 kDa and an acidic isoelectric point. At least seven IgE-binding regions have been identified for Ova, with the epitopes clustering at the amino and carboxyl ends of the allergen (30). In a study by Bernhisel-Broadbent et al. (28), ovomucoid was found to be more important than purified ovalbumin by skin prick test and RAST in a group of 18 children with egg allergy. Although previous studies had shown ovalbumin to be the major allergen, these studies demonstrated ovomucoid contamination in the ovalbumin preparation.

Other egg proteins that have allergenic activity include ovotransferrin (Gal d 3 or conalbumin), an acidic 77 kDa protein that has antimicrobial activity and iron-binding properties. Lysozyme (Gal d 4) has a low molecular weight of 14.3 kDa and has been considered both a major and minor allergen depending upon the study reporting data. Apovitellin, ovomucin, and phosvitin have been identified as minor egg allergens. The carbohydrate portion of the glycoproteins in eggs does not appear to have a primary role in specific IgE binding. Both B- and T-cell epitopes have been mapped for ovalbumin and ovomucoid.

C. Peanuts

In the United States several varieties of peanuts are grown, including the Virginia, Spanish, and runner, all belonging to the family Leguminosae. Peanut proteins are classified into albumins (water-soluble) and globulins (saline-soluble). Globulins are further divided into two major fractions, arachnin and conarachnin, often referred to as legumins and vicilins, respectively. Arachnins exist as macromolecules of 600 kDa and readily dissociate into 340–360 kDa dimeric structure and monomers of 170–180 kDa. Ultracentrifugation has been used to divide conarachnin into 2S and 8.4S fractions.

The major allergenic proteins have been identified, cloned, and expressed recombinantly and the IgE-binding and T-cell epitopes mapped. Ara h 1 is a

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glycoprotein member of the vicilin family of seed storage proteins or a conarachnin with a molecular weight of 63.5 kDa and an isoelectric point of 4.5 (31). Ara h 2 is a 17.5 kDa conglutin seed storage protein with an isoelectric point of 5.2 (32). Ara h 3, a minor allergen based upon frequency of IgE binding in the peanut-sensitive population, is a 60 kDa glycinin seed storage protein (33). All three allergens contain multiple IgE-binding linear epitopes identified by synthesizing overlapping peptides representing the entire allergen (33–35). The epitopes of Ara h 1 and 2 have been modified by systematically substituting the amino acids at each position to identify the specific amino acids that would prevent or drastically reduce IgE binding (34–36).

Hourihane et al. (37) studied 14 peanut-allergic subjects in a DBPCFC with 10 μg to 50 mg peanut protein in which eight of the subjects had some kind of reaction. As little as 100 μg of peanut protein evoked symptoms in some peanut-allergic patients with one subject having a systemic reaction to only 5 mg of peanut protein. Two other patients had mild objective reactions to 2 and 50 mg, while five others reported mild subjective reactions to doses between 5 and 50 mg.

D. Soybeans

Soybeans represent one of the most important nutrient sources of the legume family. It is one of the four food allergens (in addition to milk, egg, and peanut) accounting for nearly 90% of hypersensitivity reactions in food-allergic patients with atopic dermatitis. Soybean dust also produces important aeroallergens, causing incidents of asthma in occupational settings—soybean mill workers, harbor, workers animal feed workers, and bakers. Allergenic sources of processed foods include tofu, miso, soybean sauce, lecithin and oil, infant formulas, and transgenic soybeans.

Soybeans, like peanuts, are legumes that have multiple allergens (38). Legumes (peanuts and soybeans) and tree nuts are seeds whose storage proteins represent more than 10% of total protein. Seed storage of dicotyledon plants contains two major protein classes, globulins and albumins, which are distinguished on the basis of solubility. Globulins, which are soluble in high-salt buffers, have sedimentation coefficients of 7S and 12S. They are typically characterized by high levels of arginine, glutamine, and asparagine, which provide a source of nitrogen for developing seedlings and do not usually show enzymatic activity (39). For this reason they are the most likely seed allergen candidates to develop mutations in amino acid sequences that fail to bind IgE using recombinant gene technology for the development of hypoallergenic plants.

The albumins are a more diverse group of proteins, usually soluble in water with sedimentation coefficients of approximately 2S, and are rich in cysteine, glutamine, and arginine. This group of proteins have seed-storage function as well as biological activity (proteases, trypsin, and α-amylase activity) and are less

likely candidates for gene manipulation for hypoallergenic plant development. Distinctive among this group are a number of 2S seed-storage proteins from different plants that have a limited but distinct sequence homology and have been grouped into a 2S superfamily of related sulfur-rich seed proteins. The position of cysteine residues relative to each other appears to be completely conserved, suggesting that tertiary structure constraints imposed by disulfide bridges dominate sequence conservation. Allergens represented in this group include the 2S proteins of the castor bean, rapeseed, mustard seed, Brazil nut, and pea seed, all having the same structural organization and consisting of two subunits of different sizes linked by disulfide bridges.

Aeroallergens Gly m 1 and 2 and the Kunitz-trypsin inhibitor are limited primarily to the soybean hull. Soybean seed proteins, Gly m Bd 30K, β-conglycinin, and more recently profilin, are the predominant ingestive allergens.

E. Wheat

Wheat is one of the foods most commonly responsible for allergic reactions in children and adults. Hypersensitivity reactions to ingested wheat protein sources typically occur within an hour and include cutaneous, gastrointestinal, and/or respiratory symptoms. These reactions are clinically distinct from other adverse reactions to wheat protein, including baker's asthma, an inhalant IgE-mediated reaction to wheat and other cereal grain flours; celiac disease, a non–IgE-mediated enteropathy caused by wheat gliadin; and wheat-dependent exercise-induced anaphylaxis.

Proteins from wheat as well as other cereal grains are also classified into two fractions: salt-soluble (albumin and globulin) and salt-insoluble (glutenin and gliadin) (40). Some discrepancy exists in identifying the source of major wheat allergens being either the salt-insoluble or the salt-soluble fraction. This can be explained in part by the anomalous relative mobilities in SDS/PAGE under different reaction conditions (reducing/nonreducing gels) and the reactivities of these fractionated proteins with sera from patients to wheat proteins, which indicates that the type and number of epitopes of allergens are variable among patients. However, Watanabe et al. (41) found that most wheat-allergic patients were sensitive to a low molecular weight glutenin subunit containing a number of pentapeptide repeat motifs, Gln-Gln-Gln-Pro-Pro, which was identified to be the IgE-binding epitope (42). Immunoblot analysis and N-terminal sequencing of a 15 kDa wheat protein indicated that wheat α-amylase inhibitor also represented a sensitizing allergen following ingestion as well as inhalation (43)

F. Shellfish/Fish

Allergies to fish are a common problem in fish-processing and fish-consuming communities. Fish allergens can act as both inhalant and food allergens. For example, codfish allergy is extremely common in Scandinavian countries. Gad c 1

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(formerly allergen M) of cod fish belongs to a large group of vertebrate muscle calcium-binding proteins, the parvalbumins. This acidic, pI 4.75 allergen is composed of 113 amino acids with a molecular weight of 12,328 (44) with an IgE-reactive epitope that resists cooking, digestion, and denaturation. A high amino acid sequence homology between Gad c 1 and corresponding parvalbumin of other fish species accounts for much of the allergenic cross-reactivity identified in different fish species. Five IgE-binding sites have been identified in this allergen. The carbohydrate moiety does not appear to be important allergenically.

The tropomyosins (~36 kDa) are a homologous set of allergens identified in brown shrimp (Pen a 1), greasyback shrimp (Met e 1), spiny lobster (Pan s 1), and the American lobster (Hom a 1). Tropomyosins are also present in vertebrate food sources, e.g., bony fish, beef, pork, and chicken, yet these foods are rarely allergenic. Reese et al. identified 4 peptides (13–21 amino acids in length) located at the carboxyl end of Pen a 1 (45). The peptides aligned with both conserved and nonconserved regions of tropomyosin but were not identical to vertebrate homologs, suggesting that unique amino acid sequences may be responsible for the differences in allergenicity.

G. Tree Nuts

Tree nuts, including the Brazil nut, walnut, almond, hazelnut, and cashew, can be responsible for severe food-induced allergic reactions. Like peanut-sensitive individuals, tree nut–sensitive individuals rarely become tolerant and face the accidental ingestion from the ubiquitous nature of foods containing tree nuts. Only recently has the allergen characterization of tree nuts been investigated. Clinically, patients who are allergic and have anaphylaxis to one tree nut are often allergic to several others. This has been hypothesized to be due to epitope homology between the seed-storage proteins; however, no sequence motifs have been identified.

Two major allergens, a 70 kDa heat-labile protein and a 45–50 kDa heat-stable protein, have been identified in almonds. Several different proteins, including Ber e 1, a 12 kDa high methionine 2S protein consisting of two subunits, a 9 kDa and a 3 kDa protein (46) and a 12S globulin protein (47), show strong IgE-binding capacity in Brazil nut extracts. Walnuts rank third in per capita consumption of tree nuts in the United States and have been associated with systemic IgE-mediated reactions. A cDNA clone containing an insert of 663 bp has been identified and appears to be the major walnut food allergen (48). An IgE-binding inhibition study suggested that the walnut 2S protein precursor undergoes post-translational modification into large and small subunits similar to the castor bean, cottonseed, mustard seed, and the Brazil nut seed storage protein allergens, making it a member of the 2S superfamily of seed storage proteins.

Data from a study to determine the allergenicity of gourmet nut oils indicate a wide variability in IgE binding from pooled serum to various brands and types

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of nut oil extracts (49). Conclusions of the study indicated that several brands of minimally processed nut and peanut oils could contain enough antigen/allergen to pose a threat to hypersensitive individuals. High temperature under vacuum generally correlated with both protein content and in vitro IgE binding.

V. Diagnosis of Food Allergy

The wide range of immunological and physiological mechanisms that are responsible for the clinical symptoms of adverse reactions to foods make it difficult to rely on any single test to identify the specific component responsible for the reaction. Atopic dermatitis is typically the first manifestation of a child prone (genetic predisposition) to develop atopic reactions, with 5% of all AD individuals developing in the first year of life and 80% by 5 years of age. Approximately 80% of children with AD develop asthma or allergenic rhinitis, with many losing their AD with the onset of respiratory allergy. In the pathogenic role of food allergy in presenting symptoms, IgE-mediated food-induced respiratory symptoms are uncommon. However, they can be observed in young children with AD and in a small subset of patients with asthma.

A. Clinical Syndromes

Food allergies commonly begin in the first 2 years of life, occurring in about 8% of infants, with only 1–2% persisting or occurring in adulthood. Most adverse reactions to foods that occur in adults are not due to allergy but are intolerance reactions. The clinical syndromes of food allergy can therefore be categorized as systemic/intestinal anaphylaxis, oral allergy syndrome, and exercise-induced food anaphylaxis.

Systemic/Intestinal Anaphylaxis

This syndrome is characterized by laryngeal edema (swelling of the throat) that leads to smooth muscle constriction of the smooth muscles in the airways and failure to breathe accompanied by a loss in blood pressure and ultimate unconsciousness. Gastrointestinal symptoms include nausea, vomiting, and diarrhea. Respiratory symptoms include sneezing, nasal congestion, rhinorrhea (runny nose), conjunctivitis, (itchy, watery, red eyes), and asthma (wheezing). Fatal food-related anaphylaxis appears to be more common in individuals with underlying asthma (11).

Oral Allergy Syndrome

Epidemiological studies report a prevalence of oral allergy syndrome, (OAS) ranging from 23 to 47% in populations of patients suffering from pollenosis (50,51). Symptoms consist of itching, tingling, or swelling of the tongue, mouth,

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or throat occurring while or immediately after eating a food. This reaction usually occurs in some but not all pollen-allergic individuals who eat certain fruits and vegetables. For instance, individuals with ragweed pollen allergy may react to bananas and melons. Those with birch pollen allergy may not be able to tolerate apples, carrots, celery, hazelnut, kiwi, pear, or potato. Grass pollen–allergic individuals may have problems eating celery or peaches. The relationship of the pollen to the food indicates that they have similar chemical structures or appear in a food source (e.g., pollen-contaminated honey) recognized by the person's immune system.

Exercise-Induced Allergy

Although the pathogenesis of exercise-induced allergy (EIA) is poorly understood, biopsy studies have demonstrated mast cell degranulation after exercise. Patient's with EIA who have specific foods as coprecipitating factors invariably have positive skin test reactions to these foods, thus specific IgE mast cell sensitization is indicated. A potential dysfunction in the autonomic nervous system has also been hypothesized. Differential diagnosis includes cholinergic urticaria.

Two subsets of food-dependent EIA exist: nonspecific and food-specific, where the anaphylactic episode is triggered following a meal or specific food, respectively. The majority of reports described involve trained athletes; however, it is unclear whether the magnitude and frequency of exercise are independent risk factors. Intervals between eating the food source and symptomatic exercise range from minutes to hours.

Food-Dependent, Exercise-Induced Anaphylaxis

This can be a serious life-threatening relationship until identified by the individual. Eating certain foods and then exercising within 4 hours results in hives, wheezing, and even shock. Exercising alone or eating the food and not exercising does not cause the syndrome. Foods frequently implicated are celery, carrots, apples, shrimp, oysters, and chicken. A similar reaction occurs in some individuals who are allergic to nonsteroidal anti-inflammatory drugs such as aspirin and ibuprofen.

Atopic Dermatitis

Atopic dermatitis (AD) is a chronic disorder that begins in children characterized by extreme pruritis, a chronically relapsing course, and an association with asthma and allergic rhinitis. Approximately one-third of children who have atopic dermatitis has a food allergic reaction. In food challenges, skin symptoms that are provoked include a pruritic, erythematous, morbiliform rash that develops in sites with a predilection for atopic dermatitis. Urticarial lesions are not uncom-

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mon. On challenge, many patients may present with gastrointestinal and respiratory symptoms as well. In children with AD and food allergy, the clinical correlation with asthma appears to be high. James et al. (52) reported that 17% of children with AD experienced wheezing during a positive food challenge.

B. Diagnostic Tests

The diagnosis of food allergy requires a careful search for the possible causes. Confirmation of the cause with supporting tests includes specific tests for allergen-specific IgE (i.e., prick skin tests, radioallergosorbent tests) and, in some cases, oral food challenges. The initial evaluation should begin with a thorough history and physical examination considering a broad differential diagnosis, including metabolic disorders, anatomical abnormalities, malignancy, pancreatic insufficiency, nonimmunological adverse reactions to foods, and other disorders that could lead to similar symptoms. Important elements of a good history include (1) identifying the suspected food, (2) identifying the quantity of ingested food, (3) determining the length of time between ingestion and the development of symptoms, (4) an accurate description of the symptoms, (5) any previous occurrences, (6) involvement with other factors (e.g., exercise or cold), and (7) time from any previous reactions.

Skin Testing

As in standard methods for determining allergy, the in vivo prick puncture skin test with food extracts can be used as a screening test to identify or, more importantly, to exclude the responsible foods. The patient must be off antihistamines for at least 72 hours. A bifurcated needle or lancet is used to puncture the skin through a drop of a commercially glycerinated extract of food, an appropriate positive control (histamine) and negative control (saline-glycerin). A local wheal-and-flare response indicates the presence of a food-specific IgE. A wheal diameter of >3 mm indicates a positive response. Unfortunately, the positive predictive value is on the order of only 50%. Thus, a positive skin test cannot be considered proof of a clinically relevant hypersensitivity. The negative predictive value is very high (on the order of $>90\%$) and virtually rules out IgE-mediated food allergy. Intradermal skin tests are contraindicated because they give an unacceptably high false-positive rate as well as increased risk to the individual (53,54).

Skin testing should be considered an excellent means of excluding IgE-mediated food allergen, but it is only suggestive of clinical food allergy. Exceptions can include the following: (1) IgE-mediated sensitivity to several fruits and vegetables is frequently undetected with commercial extract preparations as they represent very labile proteins; (2) children less than 1 year of age may have IgE-mediated food allergy without a positive skin test; (3) children younger than 2 frequently have smaller wheals caused by lack of skin reactivity; and (4) a posi-

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tive skin prick test may be registered for food ingested in isolation, which provokes a serious anaphylactic reaction and may not be considered diagnostic.

RAST

As with skin testing, RAST testing to specific foods is very reliable in ruling out an IgE-mediated reaction, but a positive result has a low predictive value. In vitro tests for IgE are generally less sensitive than skin tests; however, using highly sensitive assays (CAP system FEIA) for food-specific IgE antibody correlates with clinical reactivity to certain foods (milk, egg, peanuts, fish) (16). In these studies, levels of >15 kU/mL of specific IgE to peanut have a greater than 95% predictive accuracy of predicting a positive food challenge. At present, a 95% positive predictive value of allergen-specific IgE in kU/L of CAP-RAST results are limited to the following major food allergens: milk (32 kU IgE/L), egg (6 kU IgE/L), peanut (15 kU IgE/L), and fish (20 kU IgE/L) (16). Because of extensive in vitro cross-reactivity, a 50% best positive predictive value for soy was determined to be 65 kU IgE/L and wheat at a 75% predictive certainty with 100 kU IgE/L. Measurements of IgG_4 antibody, provocation-neutralization, cytotoxicity, applied kinesiology, and other unproven methods are not useful (55).

Oral Food Challenge

The gold standard for diagnosing food allergy is the double-blind, placebo-controlled food challenge (54,56,57). Patients are required to avoid the suspected food for at least two weeks, antihistamine therapy is discontinued according to the elimination half-life of the specific medication, and doses of asthma medications are reduced as much as possible. After intravenous access is obtained, graded doses of either challenge food or a placebo food are administered hidden either in another food (orange juice) or in opaque capsules. Doses begin at 125 mg and double every 15–60 minutes. If 10 g of the challenge material is tolerated, the test must be confirmed by an open challenge. Medical supervision and immediate access to emergency medications, including epinephrine, antihistamines, steroids, and inhaled β-agonists, and equipment for cardiopulmonary resuscitation are necessary. During the challenge, the patient is assessed for changes in skin, gastrointestinal tract, and respiratory symptoms. Challenges are terminated when a reaction becomes apparent with emergency medications provided as needed. Patients are observed for delayed reactions. Negative challenges are always confirmed with an open feeding of a larger, meal-sized portion of the suspected food. Oral challenges are not performed in patients with a clear history of reactivity or a confirmed severe reaction.

Double-blind, placebo-controlled food challenge (DBPCFC) data for food allergies in the United States indicate that only seven foods account for nearly 95% of the reactions (58). The foods reported that cause adverse reactions in

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children include egg (25%), peanuts (24%), milk (23%), tree nuts (10%), soy (6%), fish (3%), and wheat (2.5%) (59).

VI. Cross-Sensitivity Between Foods

Allergenic reactions to food are usually very specific. Although patients frequently have positive skin prick tests and RAST to several members of a botanical family or animal species, indicating immunological cross-reactivity, the frequency of patients experiencing intrabotanical or intraspecies symptoms is quite variable. For example, extensive immunological cross-reactivity has been demonstrated with SPT, RAST, and immunoblot analysis in most patients with a legume sensitivity. Although this wide in vitro cross-reactivity between members of the Leguminosae family has been observed, on verifying clinical reactivity to each member by DBPCFC, only 5% of the patients reacted to more than one of the legumes despite a positive RAST for almost all legumes (28). Examples of clinical cross-reactivity include peanuts with other legumes, 3–5%; cereal grains with other grains, ~20%; cow's milk to goat's milk 90% or to beef 10%; beef to lamb 40%. Similarly, cross-reactivity among fish-sensitive individuals is quite limited. However, in RAST-inhibition experiments with sera from children allergic to fish, including cod, bass, dentex, eel, sole, and tuna, were shown to have cross-reacting allergens (60). Consequently, the practice of avoiding all foods within a botanical family when one member is suspected of provoking an allergic symptom appears to be unwarranted and puts the patient at risk for malnutrition.

Cross-reactivity between certain foods and pollens and between food and latex products has been well documented. The birch protein, Bet v 1; plant profilin, Bet v 2; and mugwort pollen allergen, Art v 1, have all been implicated as major cross-reactive allergens in pollens, fruits, and vegetables (61–63). In the northeastern United States, cross-reactivity between birch pollen and a variety of fruits and vegetables, including apples, carrots, and potatoes, is common. The most frequent complaints include apples, cherries, peaches, and pears in individuals with spring pollenosis. Ragweed, grass, and minor weeds have also been associated with food sensitivities. Food allergy caused by crustacea and mollusks has been described in patients with house dust mite allergy (64–66). Tropomyosin, the highly conserved muscle protein, has been identified as cross-reactive in house dust mite and shrimp as well as several other invertebrates (64,65). Crespo et al. (67) demonstrated a cross-reactivity of IgE-binding components between boiled Atlantic shrimp and German cockroach in sera from 89 patients.

Cross-reactivity is also common between latex and a variety of foods, mostly fruits. Presentation may include cutaneous, urticaria, eczema, and dermatitis; airborne—rhinitis, conjunctivitis, and asthma; and mucosal leading to ana-

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phylaxis. Pediatric groups at high risk for IgE-mediated latex allergy includes children with spina bifida and those with recurrent urinary tract contact with latex.

While all of these antigens have a contributory role in allergic asthma, the clinical significance of such cross-reactivity remains to be determined. IgE directed to cross-reactive carbohydrate determinants (CCD) with no clinical significance in subjects with multiple positive RAST value (68) might also explain some cross-reactivity findings. IgE antibodies are able to recognize unrelated glycoproteins to specific allergens if the glycoprotein contains N-linked $\beta 1 \rightarrow 2$ xylose and $\alpha 1 \rightarrow 3$ fucose residues common and unique to plant glycoproteins. The involvement of these xylose- and fucose-containing complex glycans in allergenic responses has been said to be underestimated. These glycans can provide a structural basis that may explain the cross-reactivities often observed between pollen, vegetable, and insect allergen (69). Thus, in vitro immunological findings need to be correlated with the clinical reactions to foods in each patient before prescribing an elimination diet free of all related or unrelated food allergens sharing IgE reactivity. The search for molecular support for clinical findings of cross-reactivity has not yet produced any definitive evidence.

VII. Prevention

Systematically, allergy prevention can be directed into at least three potential strategies: (1) primary prevention that inhibits IgE and other immunological sensitization, (2) secondary prevention that abrogates disease expression subsequent to immunological sensitization, and (3) tertiary prevention that suppresses symptoms after and despite disease expression (70).

Despite the sophistication of our current understanding of immune responses, it is still uncertain which changes induced by conventional immunotherapy are responsible for symptomatic improvement. This is even more evident in food allergy than in inhalant allergy. Potential routes of food exposure that lead to specific food IgE antibody include (1) the placenta, prenatally, (2) breast milk during lactation, (3) formula feeding, (4) solid food feedings, and (5) accidentally or covertly through food allergens in airborne droplets, floor dusts, and by caretakers. As a result, elimination diet regimens have been the mainstay therapy to prevent allergenic food exposure to prevent sensitization, often with mixed or conflicting results.

As in other allergic diseases, both patient factors and external factors contribute to fatal food-induced anaphylaxis (71). Patient factors include failure to inquire about ingredients in foods prepared by others, failure to appreciate the dose-response nature of food allergens, and denial or minimalization of symptoms based on previous nonfatal reactions. Patients may fail to carry or use injectable epinephrine or may mistakenly rely on oral antihistamine therapy for

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control of symptoms. In adolescents or young adults, drug or alcohol use may impair judgment and enhance absorption of food. External factors include a lack of awareness of the life-threatening nature of food allergy by restaurant or lunchroom personnel and inadequate labeling of packaged foods. Most importantly, lack of emergency resuscitation equipment and the inappropriate use of emergency medication by medical personnel or paramedical personnel may be contributing factors.

Careful repeated reading of food labels is critical. Consumers must learn to recognize the various names indicating the presence of a food to which they are sensitive. Products may list casein, caseinate, or whey rather than milk as the ingredient. Vague labeling such as ''natural flavoring'' or ''hydrolyzed protein'' should be interpreted carefully. Recent studies have found large amounts of milk protein in ''nondairy'' products, including canned tuna that listed only tuna, water, and salt as the labeled ingredients (72). Greater prudence with ingredient labeling of foods that may contribute to potential food sensitivities will require a commitment by industry to inform the consumer of the presence of ingredients that can cause adverse reactions in susceptible individuals. Susceptible individuals must become aware of and understand the terminology associated with labeling and avoid potentially life-threatening reactions.

Breast-feeding and delayed introduction of highly allergenic foods are common approaches for attempting to prevent food allergies in infants considered at high risk. However, it is unclear whether breast-feeding alone can prevent the development of food allergies. The authors of a retrospective study in Arizona suggested that breast-feeding might have reduced the likelihood of infants developing viral infections, a potential cause of bronchospasm, rather than actually preventing allergic sensitization (73). A multipronged approach to prevent food hypersensitivity in offspring of high-risk families included placing women in the last trimester on rigorous elimination diets, breast-feeding for at least 6 months, withholding foods highly allergenic from the infant for 6–18 months, and avoidance of mites, mold, cigarette smoke, and animals in the environment (74). The results were discouraging, with virtually no difference seen in the prevalence of food allergy, atopic dermatitis, rhinitis, and asthma. In a similar study in England, pregnant mothers rigorously avoided dairy products, eggs, fish, and nuts and practiced respiratory environmental controls to prevent sensitization in utero. Infants from breast-feeding mothers in this study demonstrated at age 2 a significant reduction in the incidence of allergy and atopic eczema (75). Overall, the results of these studies suggest that environmental control of allergen sensitization, breast-feeding with rigorous elimination diets of known food allergic–sensitizing foods, and elimination of highly allergenic foods from the child's diet for 6–18 months may be beneficial.

Strict avoidance remains the primary therapy for children and adults with food allergies. A variety of excellent resources are available for parents and

health-care professionals in their efforts to control food allergies. The Food Allergy Network is a particularly useful resource (Food Allergy Network, 4744 Holly Avenue, Fairfax, VA 22030-5647; telephone (703) 691-3179, Fax (703) 691-2713).

VIII. Control

Although the point of control is prevention of sensitization in the first place, control mechanisms for the allergic individual are still the best alternative. By controlling the immune system, individuals can control their allergies to foods. The following highlights, adapted from RH Schwartz's Allergy & Asthma Rochester Resource Center web page, identity the significant areas in the management/control of food allergy (http://www.aarrc.com; http://www.foodallergy.org).

1. *Don't eat the food to which you are allergic*! This is the principal of management and is termed ''avoidance.'' Food-allergic individuals need to carefully read all labels of commercially prepared foods and be aware of the ingredients in which food allergens may be hidden. Examples include the presence of whey (milk proteins) in breads and the use of peanut butter in Chinese egg rolls and chili. It is up to the individual or parent to alert school personnel, doctors, nurses, and dieticians of the incriminating food so that avoidance can be a part of the daily routine.
2. *Immediate treatment*. Immediately administer an injection of epinephrine (adrenalin) if symptoms are initially severe or are mild and get worse quickly. Injections last only half an hour, so it is important to get to emergency shock treatment facilities as soon as possible for follow-up treatment and monitoring. Food-allergic individuals, and the personnel surrounding them, need to familiarize themselves with the use of EpiPens. The most convenient epinephrine devices at home or at school are the autoinjectors: EpiPen or Epi-EZ-Pen (0.3 mg of epinephrine 1:1000) and for the child under age, EpiPen Jr., or Epi-EZ-Pen Jr. (0.15 mg of epinephrine 1:2000). Remember: an EpiPen at home or in the medicine cabinet is not going to help if you have a reaction at a restaurant, friend's home, dormitory, or at school.
3. *Eat something else*! Hypoallergenic formulas are available for infant milk formulas that are prepared from soybeans or rice. In this particular instance, it is the individual's responsibility to be aware of potential hidden sources of the allergen. Food product awareness is essential, as the presence of cow's milk protein can include ''casein,'' ''whey,'' ''lactalbumin,'' ''caramel color,'' and ''nougat.'' Other indications of hidden allergens include egg substitutes that contain chicken egg pro-

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teins. Similarly, contaminants may pose a problem: spatulas used to serve cookies with both peanut butter and peanut-free cookies have been shown to cause reactions. Contamination can occur when common equipment is used in the manufacture of candies.

4. *Don't test for tolerance*! Although it is common to outgrow many of the food allergies, it is extremely dangerous to self-administer small amounts of the incriminating food to determine if the allergy no longer exists. Consult an allergist specialist who can assess the level of allergy with skin and/or blood tests and ultimately a food challenge under controlled circumstances before consideration of ''immunological tolerance'' has been attained.

IX. Treatment

Strict elimination of the responsible food allergen and/or source is the only proven therapy currently available for prevention of food hypersensitivity reactions. A properly managed elimination diet, excluding only foods proven to evoke an allergic response, can lead to resolution of food allergy and avoid potential malnutrition and other undesirable outcomes. However, elimination diets for foods such as milk, egg, wheat, soy, and peanut can be very difficult to adhere to without accidental ingestion of the allergen in hidden sources. In addition, with important foods like milk, there is the risk of negative effects on the nutritional status of the allergic individual. For example, calcium intake below the recommended daily allowance was found in a population of 58 patients diagnosed with cow's milk allergy (76).

Conventional immunotherapy involves the injection of increasing doses of allergen extract until maintenance level is achieved. In this form of therapy, high doses of allergen are suggested to encourage a shift in antigen-presenting cells from B cells to macrophages and monocytes with a concomitant shift from the Th2-type response to a Th1-type response (77). Unfortunately, conventional immunotherapy has not proven beneficial for food allergy. In studies using conventional rush and maintenance immunotherapy for peanut-sensitive individuals, maintenance immunotherapy with aqueous peanut extracts was accompanied by a high rate of systemic reactions (78,79). Although the authors were able to clearly demonstrate that injections of peanut extract significantly increased the tolerance of anaphylactically sensitive subjects to ingested protein, half of the subjects could not be maintained because of repeated systemic reactions to the injections. The authors concluded that the high rate of systemic reactions made this form of treatment with the available peanut extracts unacceptable.

With current research, it may be possible to determine common motifs involved in IgE regulation and induction that will prove to be beneficial in food

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therapy regimens. Areas of investigation include plasmid naked DNA vaccines, altered T- and/or B-cell epitopes, and oral antigen vaccines to divert the GALT from a Th2 to Th1 response. Other potential therapies include competitive inhibition of allergen presentation by developing antibodies to TCR v regions and soluble peptide inhibition.

An appropriate animal model with similar comparative aspects of form and function relative to the human allergic response to elucidate the pathogenesis and mechanisms of food allergy is a current area of intense investigation. Ermel et al. (80) and Fick (81) have established an inbred colony of high immunoglobulin E–producing dogs that when immunized subcutaneously with food antigen extracts in alum developed clinical manifestations of food allergy after oral challenge with food antigen. Skin tests were consistently positive, and mucosal changes involving swelling and erythema of the gastric mucosa suggest that this model may be useful as an animal model for food allergy. The Brown Norway rat has been used for ovalbumin antigen-dose response production of an antigen-specific IgE–induced antibody response through the oral route (82). To promote IgE production, the adjuvant carrageenan was administered once a week by an intraperitoneal route. Knippels et al. (83) also used the brown rat model to study oral sensitization to food proteins. Upon oral challenge with OVA of both orally and parenterally sensitized animals with or without adjuvant, gut permeability was shown to be increased, and local effects were observed in all animals, whereas systemic effects were observed at a low frequency. Both IgG and IgE responses as well as specific T-cell–mediated hypersensitivity (DTH) to ovalbumin could be demonstrated via the enteral route without the use of adjuvants.

Oral allergen-gene immunization to modulate peanut antigen–induced murine anaphylaxis has been used to generate immunological protection in a murine model (84). Oral administration of DNA nanoparticles, a complex of plasmid DNA and chitosan, resulted in transduced gene expression of the allergen in the intestinal epithelium and produced secretory IgA and serum IgG2a. This study suggests a prophylactic measure for treating food allergy; however, it requires the identification of individuals at risk and does not support or address the treatment of ongoing IgE-mediated responses. A plasmid cDNA-based gene construct, pAra h 2, suggested that pDNA immunization in mice was strain specific showing significant increases in IgG2a but not IgG1 or IgE (85). In other studies, Li et al. (86) investigated a murine model of IgE-mediated cow's milk hypersensitivity by intragastric sensitization plus cholera toxin with significant cow's milk specific IgE antibody levels that may be useful for exploring new therapeutic approaches to cow's milk allergy. Miyajima et al. (87) suggested that in relation to the pathophysiological changes associated with active anaphylaxis in the mouse, the mortality associated with this response could be mediated largely by IgA sub 1 antibodies and Fc gamma RIII. A murine model was used to show an important correlation between Th2 cytokine, IgE production, and histamine release by intra-

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peritoneal administration of ovalbumin on dried aluminum hydroxide but failed to reflect the gastrointestinal clinical situation in patients with food allergy (88). Transient immune responses have been shown with soybean allergens in the pig; however, it remains to be determined if IgE is the responsible antibody (89)

There is growing evidence emerging for nerve involvement in mast cell degranulation and induction of digestive disturbances by antigen challenge. For example, mast cell degranulation was induced by a Pavlonian reflex in egg albumin–sensitized rats (90), and the neurotoxin tetradotoxin was reported to inhibit the increase in intestinal permeability induced by egg albumin challenge in sensitized rats (91). Recently, Gay et al. showed that in guinea pigs sensitized to cow's milk treated with neurokinin receptor antagonist, both IgE and IgG serum titers were lower (92). These interesting animal studies may open new areas of investigation into the clinical manifestations in the gastrointestinal system and food-allergic reactions.

Although these animal models are available to study food allergy and allergenicity of food proteins, no fully validated enteral animal models mimicking the human allergic response are yet available to determine allergic mechanisms in food allergy. It remains to be demonstrated in an animal model that an ability to shift dangerous allergic responses to a more tolerant response is possible.

X. Genetically Engineered Crop Plants

Several biotechnological techniques can be applied to reduce or eliminate the allergenicity of proteins identified as food allergens. These techniques include chemical and physical treatments and several biotechnological methods, such as enzymatic and microbiological modification of the proteins including recombinant DNA technology. Useful approaches for the preparation of hypoallergenic food include introducing structural changes that enhance digestibility. It has been demonstrated that treatment with thioredoxin reduces allergenicity of wheat proteins and lactoglobulin (93,94). These methods are often labor intensive and cost prohibitive.

Food biotechnology is a precise method for enhancing the beneficial traits of plants, animals, and microorganisms. This technology has the potential to assist the agri-food industry in achieving key objectives including improvements in nutritional characteristics, improved taste, elimination of undesirable traits, extension of shelf life, decreased use of additives, reduced processing costs, reduced energy consumption, and lessening of environmental impact from food processing (95).

Successfully genetically engineered crop plants that have traits including insect resistance, modified starch, herbicide tolerance, modified oils, disease resistance, male sterility, and delayed ripening have been introduced into the mar-

ketplace. Most traits introduced into crops result in the expression of one or a few modified proteins. In some cases, genes are introduced that turn off other genes and no new protein is introduced. The increased allergenic potential of foods developed from genetically modified organisms targets three levels (96): (1) the plant from which the gene comes, (2) the protein induced by this gene, by comparing it to known allergenic food proteins and others, and (3) modification of the composition of the host plant, which may eventually affect the allergenicity of these plant's proteins. To deal with the input of genetically modified plant crops into the marketplace, the International Food Biotechnology Council in conjunction with the Allergy and Immunology Institute of the International Life Sciences developed a decision-tree approach for the assessment of allergenicity of genetically modified food (97). The strategy focuses on the source of the gene, the sequence homology of the newly introduced protein to known allergens, the immunochemical reactivity of the newly introduced protein with IgE from blood serum of sensitive individuals, and the physicochemical properties of the newly introduced protein. Prior to introduction into the market, solid phase immunoassay, skin prick tests, and finally a DBPCFC test to determine the level of allergenicity will be used to assess gene products from known allergenic genetic material. If the gene source is derived from a nonallergenic source, sequence similarity and stability to digestion/processing will be taken into account prior to consultation with regulatory agencies prior to release to the market. For further information, the reader is referred to several review articles regarding the assessment of allergenicity of genetically modified foods (98–100).

An example of improving the nutritional quality by transgenic methods of a food crop is found in the legume family. The nutritional quality of legumes, for both humans and animals, is compromised by a deficiency of methionine within the protein fractions of the seeds. Attempts to manipulate the balance of essential amino acids such as methionine in legume plants by traditional plant breeding methods have failed due to decreased yield or quality. The introduction of genes encoding sulfur-rich proteins (2S albumins) from other plants through recombinant DNA techniques proved to be a more promising strategy for improving the nutritional quality of sulfur-deficient seeds without adversely affecting agronomic performances. The 2S albumin from the Brazil nut is one of the richest sulfur protein sources described in nature (~30%). Its high stability with no known biological activity in the plant made it a logical choice for gene expression in soybean, tobacco, oilseed rape, beans, and other legumes to improve the nutritional quality of these seeds. Nordlee et al. (46), recognizing that the Brazil nut was allergenic to sensitive individuals, applied the in vitro steps of the decision-tree assessment to determine the potential allergenicity of the modified plant crop. Using immunoassay, IgE immunoblot analysis, and skin prick tests, they were able to identify the 2S methionine-rich protein as the major allergen of Brazil

nut, Ber e 1. Consequently, the transgenic soybean product was withdrawn from consideration as a nutrient source.

An antisense RNA strategy has been applied to repress the allergen expression in maturing rice seeds (101). Using immunoblot and ELISA analysis of seeds by monoclonal antibody to the 16 kDa allergen showed that the allergen content from several transgenic rice plants was markedly lower than that of seeds from the parental wild-type rice. However, it is still uncertain whether this hypoallergenic rice seed is tolerable for patients sensitive to rice.

With the advent of recombinant DNA technology, plant breeders now have extended the range of biological material from which genes can be accessed and gained insight into genome organization and gene structure and the nature and function of the proteins that genes encode. The technology affords unique opportunities to identify the individual components of foods that may cause allergies and to remove them from the food, or change them, so that the food can be consumed safely. The assessment tree for allergenicity will provide the safety measures necessary to provide the consumer with a safe product that will not introduce allergenic food sources to the market.

XI. Epidemiology/Case Studies

In a cohort of more than 1000 children followed from birth to age 18, the strongest predictor for childhood airway hyperresponsiveness and symptoms of asthma was family history, especially of maternal asthma and atopy (102). In longitudinal studies, allergies to cow's milk and egg usually resolve early in life; 85% of children with cow's milk allergy in the first 2 years of life are tolerant of milk by age 3 (17), and up to 80% of infants with egg allergy are tolerant of egg by age 5 (103,104). Follow-up studies of a population-based group of Danish children with cow's milk allergy suggests that resolution of the allergy is unusual if it has not occurred by age 5 (105). The mechanism of resolution remains unknown.

In a questionnaire survey of 122 patients with acute allergic reactions to peanut and tree nuts, Sicherer et al. (106) reported that allergic reactions to peanuts occur early in life. Peanut- and tree nut–allergic reactions coexist in one third of peanut-allergic patients and frequently occur on the first known exposure, often requiring emergency medical treatment. In a related nationwide study, a cross-sectional random digit dial survey of 4,374 participating households representing 12,032 individuals, a corrected prevalence among the general U.S. population for allergen reactions to peanuts and tree nuts was 1.1% (95% CI 1.0–1.4%) (107). In a report by Foucard and Yman (108), the results of a 3-year study in which physicians were asked to report fatal and life-threatening reactions caused by foods, peanut, soy, and tree nuts seemed to have caused 45 of 61

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reactions reported. Four youngsters who died from soy anaphylaxis with asthma were severely allergic to peanuts but had no previously known allergy to soy. Interestingly, a rather symptom-free period for 30–90 minutes between early mild symptoms and severe rapidly deteriorating asthma was associated with coprecipitating factors such as cold beverages, exercise, viral respiratory infections, and fear once the symptoms started to appear in these patients. The estimated amount of soy intake in the fatal cases was 1–10 g and was often obscured in hidden form such as in hamburger, sausage, meatballs, kebobs, and bread. This study further highlighted the need for continued study of the pathogenesis of soy-related fatalities and improved labeling of foods containing soy or other hidden known allergens.

Zeiger et al. (109) determined the prevalence of soy allergy in IgE-associated cow's milk allergy to be 14% in a cohort of 93 children aged 3–41 months. The authors concluded that soy allergy occurs only in a small minority of young children with IgE-associated CMA and that soy formula may provide a safe and growth-promoting alternative for the majority of these children.

In a more far-reaching area, a number of case reports suggest that transfer of allergen-specific IgE-mediated hypersensitivities can take place by bone marrow transplantation (110–113). More recently, the transfer of allergen-specific donor lymphocytes was believed to be the most likely mechanism for the transfer of allergy to peanuts to a patient receiving a combined transplantation of liver and kidney. Passive transfer of IgE was regarded to be unlikely; however, donor-bound IgE to recipients' mast cells and basophils could not be ruled out (114).

XII. Summary

Adverse food reactions may be secondary to food allergy or food intolerance. The food-allergic reaction results from an IgE-mediated abnormal immunological response, while food intolerance is the result of nonimmunological mechanisms. Major foods involved in children with food hypersensitivities include milk, egg, peanut, and soybeans; those in adults are principally peanuts, tree nuts, fish, and shellfish. In the diagnosis of food allergy, a positive skin test to a food extract indicates the possibility that the individual has symptomatic reactivity to that specific food. However, the overall positive predictive value is less than 50%. The double-blind, placebo-controlled food challenge remains the gold standard for identifying food hypersensitivity. Strict elimination of the responsible food allergen remains the only proven therapy to prevent fatal and near-fatal reactions in individuals at risk. Finally, although clinical investigations have characterized food hypersensitivity reactions, an understanding of the basic immunopathological mechanisms remains incomplete.

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References

1. Anderson JA, Sogn DD, eds. Am Acad Allergy Immunol/NIAID: Adverse Reactions to Foods. Washington DC: NIH Publ #84-2442, 1984:1–6.
2. Sloan AE, Powers ME. A perspective on popular perceptions of adverse reactions to foods. J Allergy Clin Immunol 1986; 78:127–133.
3. Bock SA. Prospective appraisal of complaints of adverse reaction to foods in children during the first three years of life. Pediatr 1987; 79:683–688.
4. Bernhisel-Broadbent J, Sampson HA. Cross-reactivity in the legume botanical family in children with food hypersensitivity. J Allergy Clin Immunol 1989; 83:435–440.
5. Lemanske RF, Taylor SL. Standardized extracts, foods. Clin Rev Allergy 1987; 5: 23–36.
6. King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TAE, Thomas W, WHO/IUIS Allergen Nomenclature Subcommittee. Allergen nomenclature. Int Arch Allergy Immunol 1994; 105:224–233.
7. Garcia-Casado G, Sanchez-Monge R, Chrispeels MJ, Armentia A, Slacaedo G, Gomez L. Role of complex asparagine-linked glycans in the allergenicity of plant glycoproteins. Glycobiology 1996; 6:471–477.
8. Seidman E. Food allergic disorders of the gastrointestinal track. In: Roy CC, Silverman A, Allagile D, eds. Pediatric Clinical Gastroenterology. St. Louis: Mosby, 1995:374–348.
9. Sampson HA, Metcalfe DD. Food allergies. JAMA 1992; 268:2840–2844.
10. Reiman HJ, Ring J, Ultsch B, et al. Intragastral provocation under endoscopic control (IPEC) in food allergy: mast cell and histamine changes in gastric mucosa. Clin Allergy 1985; 15:195–202.
11. Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children adolescents. N Engl J Med 1992; 327:380–384.
12. Yunginger JW, Nelson DR, Squillace DL, et al. Laboratory investigations of deaths due to anaphylaxis. J Forensic Sci 1991; 36:857–865.
13. Sampson HA, Broadbent KR, Bernhisel-Broadbent J. Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. N Engl J Med 1989; 321:228–32.
14. Romagnani S. Human Th1 and Th2 subsets. Doubt no more. Immunol Today 1991; 12:256–257.
15. Mowat AM. The regulation of immune response to dietary protein antigens. Immunol Today 1987; 8:93–98.
16. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. J Allergy Clin Immunol 1997; 100:444–451.
17. Host A, Halken S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Allergy 1990; 45:587.
18. May CD, Alberto R. In vitro responses of leukocytes to food proteins in allergic and normal children: lymphocyte stimulation and histamine release. Clin Allergy 1972; 2:335–344.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

19. May CD, Remigo L, Feldman J, et al. A study of serum antibodies to isolated milk proteins and ovalbumin in infants and children. Clin Allergy 1977; 7:583–595.
20. Gjesing B, Osterballe O, Schwartz B, et al. Allergen-specific IgE antibodies against antigenic components of cow milk and milk substitutes. Allergy 1986; 41:51–56.
21. Hefle, SL. The chemistry and biology of food allergens. Food Technol 1996; 50: 86–92.
22. Ghosh J. Malhorta GS, Mathur BM. Hypersensitivity of human subjects to bovine milk proteins. Ind J Diary Sci 1989; 42:744–749.
23. Docena GH, Fernandez R, Chirdo FG, et al. Identification of casein as the major allergenic and antigenic protein in cow's milk. Allergy 1996; 51:412–416.
24. Spuergin P, Mueller H, Walter M, et al. Allergenic epitopes of bovine alpha S1-casein recognized by human IgE and IgG. Allergy 1996; 51:306–312.
25. Nakajima-Adachi H, Hachimura S, Ise W et al. Determinant analysis of IgE and IgG4 antibodies and T cells specific for bovine alpha(s)1-caaein from the same patients allergic to cow's milk. J Allergy Clin Immunol 1998; 101:660–671.
26. Anet J, Black JF, Baker RS et al. Allergens in the white and yolk of hen's egg. Int Arch Allergy Appl Immunol 1985; 77:364–371.
27. Hoffman DR. Immunochemical identification of the allergens in egg white. J Allergy Clin Immunol 1983; 71:481–486.
28. Bernhisel-Broadbent J, Dintzis HM, Sampson HA. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared to ovalbumin (Gad d I) in children with egg allergy and in mice. J Allergy Clin Immunol 1994; 93:1047–1059.
29. Cook SK, Sampson HA. Allergenic properties of ovomucoid. J Immunol 1997; 59: 2026.
30. Honma K, Kohno Y, Saita K et al. Specificities of IgE, IgG and IgA antibodies to ovalbumin: comparison of binding activities to denatured ovalbumin or ovalbumin fragments of IgE antibodies with those of IgG or IgA. Int Arch Allergy Immunol 1994; 103:28–35.
31. Burks AW, Williams LW, Helm RM, Connaughton C, Cockrell G, O'Brien T. Identification of a major peanut allergen Ara h I, in patients with atopic dermatitis and positive peanut challenge. J Allergy Clin Immunol 1991; 88:172–179.
32. Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien TJ, Helm RM. Identification and characterization of a second major peanut allergen, Ara h II, utilizing the sera of patients with atopic dermatitis and positive peanut challenge. J Allergy Clin Immunol 1992; 90:962–969.
33. Rabjohn P, Helm EM, Stanley JS, West CM, Sampson HA, Burks AW, Bannon GA. Molecular cloning and epitope analysis of the peanut allergen, Ara h 3. J Clin Invest 1999; 103:535–542.
34. Burks AW, Shin D, Cockrell G, Stanley JS, Helm RM, Bannon GA. Mapping and mutational analysis of the IgE binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. Eur J Biochem 1997; 245:334–339.
35. Stanley JS, King N, Burks AW, Huang SK, Sampson H, Cockrell G, Helm RM, West CM, Bannon GA. Identification and mutational analysis of the immunodomi-

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

nant IgE binding epitopes of the major peanut allergen Ara h 2. Arch Biochem Biophys 1997; 342(2):244–253.
36. Shin D, Compadre C, Maleki S, Kopper R, Huang S-K, Sampson HA, Burks AW, Bannon GA. Biochemical and structural analysis of the IgE binding sites on Ara h 1, an abundant and highly allergenic peanut protein. J Biol Chem 1998; 273: 13753–13759.
37. Hourihane JO, Kilburn SA, Nordlee JA, Hefle SL, Taylor SL, Warner JO. An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized, double-blind, placebo-controlled food challenge study. J Allergy Clin Immunol 1997; 100:596–600.
38. Burks AW, Brooks JR, Sampson HA. Allergenicity of major component proteins of soybean determined by enzyme linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. J Allergy Clin Immunol 1988; 81:1135–1142.
39. Higgins TSV. Synthesis and regulation of major proteins in seeds. Ann Rev Plant Physiol 1984; 35:191–221.
40. Shewery PR, Tatham AS. The prolamin storage proteins of cereal seeds: structure and evolution. Biochem J 1990; 237:827–832.
41. Watanabe M, Suzuki T, Ikezawa Z, Ara S. Controlled enzymatic treatment of wheat proteins for production of hypoallergenic flour. Biosci Biotechnol Biochem 1994; 58:388–390.
42. Tanabe S, Arai S, Yanagihare Y, Mita H, Takahashi K, Watanabe M. A major wheat allergen has a Gln-Gln-Gln-Pro-Pro motif identified as an IgE-binding epitope. Biochem Biophys Res Commun 1996; 219:290–293.
43. James JM, Sixbey JP, Helm RM, Bannon GA, Burks AW. Wheat alpha amylase inhibitor: a second route of allergic sensitization. J Allergy Clin Immunol 1997; 99(2):239–244.
44. Elsayed S, Apol J. Immunochemical analysis of cod fish allergen M: location of the immunoglobulin binding sites as demonstrated by the native and synthetic peptides. Allergy 1983; 38:449–459.
45. Reese BG, Jeoung BJ, Daul CB, Lehrer SB. Characterization of recombinant shrimp allergen Pen a 1 (tropomyosin). Int Arch Allergy Immunol 1997; 113:240–242.
46. Nordlee JA, Taylor SL, Townsend JA, Thomas LA, Bush RK. Identification of a Brazil-Nut Allergen in Transgenic Soybeans. N Engl J Med 1996; 334:688–692.
47. Bartolome B, Mendez JD, Armentia A, Vallverdu A, Palacios R. Allergens from Brazil nut: immunochemical characterization. Allergol Immunopathol 1997; 25: 135–144.
48. Teuber SS, Dandeker AM, Petersen WR, Sellers CL. Cloning and sequencing of a gene encoding a 2S albumin seed storage protein precursor from English walnut (Juglans regia); a major food allergen. J Allergy Clin Immunol 1998; 101:807–814.
49. Teuber SS, Brown RL, Haapenen LAD. Allergenicity of gourmet nut oils processed by different methods. J Allergy Clin Immunol 1997; 99:502–507.
50. Bircher AJ, Van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

highly prevalent in patients allergic to pollen, with and without symptoms of food allergy. Clin Exp Allergy 1994; 24:367–374.
51. Pastorello EA, Incorvaia C, Pravettoni V, Ortolani C. Crossreactions in food allergy. Clin Rev Allergy Immunol 1997; 15:415–427.
52. James JM, Eigenmann PA, Eggleston PA, et al. Airway reactivity changes in asthmatic patients undergoing double blinded food challenges. Am J Respir Crit Care Med 1996; 153:597–603.
53. Bock SA, Lee WY, Remingo L, Holst A, May CD. Appraisal of skin tests with food extracts diagnosis of food hypersensitivity. Clin Allergy 1978; 8:559–564.
54. Sampson HA, Albergo R. Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. J Allergy Clin Immunol 1984; 74:26–33.
55. Terr AI, Salvaggio JE. Controversial concepts in allergy and clinical immunology. In: Bierman CW, Pearlman DS, Shapiro GG, Busse WW, eds. Allergy, Asthma, and Immunology from Infancy to Adulthood. 3rd ed. Philadelphia: Saunders, 1996: 749–760.
56. Bock SA, Sampson HA, Atkins FM, Zeiger RS. Lehrer S, Sachs M, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. J Allergy Clin Immunol 1988; 82:986–997.
57. Bock SA, Atkins FM. Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. J Pediatr 1990; 117:561–567.
58. Hide DW. Food allergy in children. Clin Exp Allergy 1994; 24:1–2.
59. Schwartz H. Allergy, intolerance, and other adverse reactions to foods. Pediatr Ann 1992; 21:1219–1225.
60. DeMantino M, Novembre E, Galli L, de Marco A, Botarelli P, Marano E, Vierucci A. Allergy to different fish species in cod-allergic children: in vivo and in vitro studies. J Allergy Clin Immunol 1990; 86:909–914.
61. Valenta R, Duchene M. Vrtala S, et al. Recombinant allergens for immunoblot diagnosis of tree pollen allergy J Allergy Clin Immunol 1991; 88:889–894.
62. Valenta R, Duchene M, Ebner C, et al. Profilins constitute a novel family of functional plant panallergens. J Exp Med 1992; 175:377–385.
63. Heiss S, Fischer S, Mueller WD, et al. Identification of a 60 kD cross-reactive allergen in pollen and plant derived food. J Allergy Clin Immunol 1996; 98:938–947.
64. Witteman AM, van den Oudenrijn S, van Leeuwen J, et al. IgE antibodies reactive with silverfish, cockroach and chironomid are frequently found in mite-positive allergic patients. Int Arch Allergy Immunol 1995; 108:165–169.
65. van Ree R, Antonicelli L, Akkerdaas JH et al. Possible induction of food allergy during mite immunotherapy. Allergy 1996; 51:108–113.
66. van Ree R, Antonicelli L, Akkerdaas JH, et al. Asthma after consumption of snails in house-dust-mite-allergic patient: a case of IgE cross-reactivity. Allergy 1996; 51:387–393.
67. Crespo JF, Pascual C, Helm R et al. Cross-reactivity of IgE-binding components between Atlantic boiled shrimp and German cockroach. Allergy 1995; 50:918–924.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

68. Kohste VL, Kagen SL, Aalberse RC. Cross-reactivity of IgE antibodies to caddis fly with arthropoda and mollusca. J Allergy Clin Immunol 1989; 84:174–183.
69. Garcia-Casado G, Sanchez-Mongue R, Chrispeels MJ, Armentia A, Salcedo G, Gomez L. Role of complex asparagine-linked glycans in the allergenicity of plant glycoproteins. Glycobiology 1996; 6:471–477.
70. Zeiger RS. Prevention of allergic disease in infancy. In: Schatz M. Claman H, Zeiger RS, eds. Asthma and Immunologic Disease in Pregnancy and Early Infancy. New York: Marcel Dekker, 1998; 761–811.
71. Yunginger JW, Squillace DL, Jones RT, Helm RM. Fatal anaphylaxis reactions induced by peanuts. Allergy Proc 1989;10:249–253.
72. Gern JE, Yang E, Evrad HM, Sampson HA. Allergic reactions to milk-contaminated ''non-diary'' products. N Engl J Med 1991; 324:976–979.
73. Wright RL, Holberg CJ, Taussig LM, Martinez FD. Relationship of infant feeding in recurrent wheezing at age 6 years. Arch Pediatr Adol Med 1995; 149:758–763.
74. Zeiger RS, Heller S. The development and prediction of atopy in high-risk children: Follow up at age seven years in a prospective randomized study of combined maternal and infant food allergy avoidance. J Allergy Clin Immunol 1995; 95:1179–1190.
75. Hide DW, Mathews S, Mathews L, et al. Effect of allergen avoidance in infancy on allergic manifestations at age two years. J Allergy Clin Immunol 1994; 93:842–846.
76. Madsen CD, Henderson RC. Calcium intake in children with positive IgG RAST to cow's milk. J Pediatr Child Health 1997; 33:209–212.
77. Umetsu DT, DeKruyff RH. Th1 and Th2 CD4+ cells in human allergic diseases. J Allergy Clin Immunol 1997; 100:1–6.
78. Oppenheimer JJ, Nelson HS, Bock SA, Christensen F. Leung DYM. Treatment of peanut allergy with rush immunotherapy. J Allergy Clin Immunol 1992; 90:256–262.
79. Nelson HS, Lahr J, Rule R, Bock A, Leung D. Treatment of anaphylactic sensitivity to peanut by immunotherapy with injections of aqueous peanut extracts. J Allergy Clin Immunol 1997; 99:744–751.
80. Ermel RW, Kock M, Griffey SM, Reinhart GA, Fick OL. The atopic dog: a model for food allergy. Lab Animal Sci 1997; 47:40–49.
81. Fick OL. Food allergy in atopic dogs. In: New Horizons in Allergy Immunotherapy. Sehon A, et al., eds. New York: Plenum Press, 1996: 1–7.
82. Atkinson HA, Johnson IT, Gee JM, Grigoriadou F, Miller K. Brown Norway rat model of food allergy: effect of plant components on the development of oral sensitization. Food Chem Toxicol 1996; 34:27–32.
83. Knippels LM, Penninks AH, Smit JJ, Houben GF. Immune-mediated effects upon oral challenge of ovalbumin-sensitized brown rat allergy model. Toxicol Appl Pharmacol 1999; 156:161–169.
84. Roy K, Mao HQ, Huang SK, Leong KW. Oral gene delivery with chitosan—DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. Nat Med 1999; 5:387–391.
85. Li X-M, Huang C-H, Schofield BH, Burks AW, Bannon GA, Kim K-H, Huang

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

S-K, Sampson HA. Strain-dependent induction of allergic sensitization caused by peanut allergen DNA immunization in mice. J Immunol 1999; 162:3–45–52.
86. Li XM, Schofield BJ, Huang CK, Kleiner GI, Sampson HA. A murine model of IgE-mediated cow's milk allergy. J Allergy Clin Immunol 1999; 103:206–214.
87. Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet J-P, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG sub 1 and Fc gamma RIII; assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG sub1-dependent passive anaphylaxis. J Clin Invest 1997; 99:909–914.
88. van Halteren AGS, van der Cammen MJF, Biewenga J, Savelkoul JFJ, Krall G. IgE and mast cell responses on intestinal allergen exposure: a murine model to study the onset of food allergy. J Allergy Clin Immunol 1997; 99:94–99.
89. Dreau D, Lalles JP, LeJan C, Toulle R, Salmon H. Hypersensitivity to soybean proteins in early weaned piglets: humoral and cellular components. In: Advances in Mucosal Immunology. J Mestecky, et al., eds. New York: Plenum Press, 1995: 865–869.
90. MacQueen G, Marshall J, Perdue M, Siegel S, Bienenstock J. Pavlonian conditioning of rat mucosal mast cells to secrete rat mast cell protease II. Science 1989; 243:83–85.
91. Crowe SE, Soda K, Staisz AM, et al. Intestinal permeability in allergic rats: nerve involvement in antigen induced changes. Am J Physiol 1993; 264:G617–623.
92. Gay J, Fioramonti J, Garcia-Villar R, Emonds-Alt X, Bueno L. Involvement of tachykinin receptors in sensitization to cow's milk proteins in guinea pigs. Gut 1999; 44:497–503.
93. Buchanan BB, Adamidi C, Lozano RM, Yee BC, Kobrehel K, Ermel R, et al. Thioredoxin-linked mitigation of allergic responses to wheat. Proc Natl Acad Sci USA 1997; 94:5372–5377.
94. Del Val G, Yee BC, Lozano RM, Buchanan BB, Ermel RM, Lee Y-M, et al. Thioredoxin treatment increases digestibility and lowers allergenicity of milk. J Allergy Clin Immunol 1999; 103:690–697.
95. Taylor SL. Food from genetically modified organisms and potential for food allergy. Environ Toxicol Pharamcol 1997; 4:121–126.
96. Astwood J, Fuchs RL. Allergenicity of foods derived from transgenic plants. In: Wuthrich B, Ortolani C, eds. Highlights in Food Allergy, Monograph Allergy. Basel: Karger. 1996: 105–120.
97. Metcalfe DD, Astwood JD, Townsend R, Sampson HA, Taylor SL, Fuchs RL. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. Crit Rev Food Sci Nutr 1996; 36:S165–186.
98. Taylor SL. Assessment of the allergenicity of genetically modified foods. AgBiotech News Inform 1997; 9:229N–234N.
99. Moneret-Voutrin DA. Modifications of allergenicity linked to food technologies. Allergie Immunol 1998; 30:9–13.
100. Bindslev-Jensen C. Allergy risks of genetically engineered foods. Allergy 1998; 53:58–61.
101. Nakamura R, Matsuda T. Rice allergenic protein and molecular-genetic approach for hypoallergenic rice. Biosci Biotechnol Biochem 1996; 60:1215–1221.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

102. Sears MR, Holdaway MD, Flannery EM, Herbison GP, Silva A. Parenteral and neonatal risk factors for atopy, airway hyperresponsiveness, and asthma. Arch Dis Childhood 1996; 75:392–398.
103. Sampson HA, McCaskill C. Food hypersensitivity in atopic dermatitis: evaluation of 133 patients. J Pediatr 1985; 107:669–675.
104. Dannaeus A, Inganaes M. A follow up study of children with food allergy. Clinical course in relation to serum IgE and IgG antibody levels to milk, egg and fish. Clin Allergy 1981; 11:533–539.
105. Host A, Halken S, Jacobsen HP, Estmann A, Mortensen S, Mygil S. The natural course of cow's milk protein allergy intolerance J Allergy Clin Immunol 1997; 99: S491.
106. Sicherer SH, Burks AW, Sampson HA. Clinical feature of acute allergic reactions to peanut and tree nuts in children. Pediatrics 1998; 102:131–132.
107. Sicherer SH, Mundoz-Furlong A, Burks AW, Sampson HA. Prevalence of peanut and tree nut allergy in the US determined by a random digit dial telephone survey. J Allergy Clin Immunol 1999; 103:559–562.
108. Foucard T, Yman IM. A study on severe food reactions in Sweden—Is soy protein an underestimated cause of food anaphylaxis? Allergy 1999; 54:261–265.
109. Zeiger RS, Sampson HA, Bock AS, Burks AW, Harden K, Noone S, Martin D, Leung S, Wilson G. Soy allergy in infants and children with IgE-mediated associated cow's milk allergy. J Pediatr 1999; 13:614–622.
110. Saarinen UM. Transfer of latent atopy by bone marrow transplantation. J Allergy Clin Immunol 1984; 74:196–200.
111. Tucker J, Barnetson RS, Eden OB. Atopy after bone marrow transplantation. Br Med J 1985; 290:116–117.
112. Walker SA, Riches PG, Eild G, et al. Total and allergen-specific IgE in relation to allergic response pattern following bone marrow transplantation. Clin Exp Immunol 1986; 66:633–639.
113. Agosti JM, Sprenger JD, Lum LG, et al. Transfer of allergen-specific IgE-mediated hypersensitivity with allogenic bone marrow transplantation. N Engl J Med 1988; 319:1623–1628.
114. Legendre C, Callait-Zucman S, Didier S. Morelon S, Bismuth H, Bach J-F, Kreis H. Brief report: transfer of symptomatic peanut allergy to the recipient of a combined liver-and-kidney transplant. N Engl J Med 1997; 337:822–824.

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Role of IgE in Allergic Bronchopulmonary Aspergillosis

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I. Introduction

Aspergillus is an ubiquitous, thermotolerant saprophyte with worldwide distribution. This dimorphic fungus derives its name from its resemblance to a brush used for sprinkling holy water (1). It is commonly found in moist soil and decomposing environment. The exposure to *Aspergillus* spores or conidia is universal and is acquired primarily via the respiratory tract (2).

This review provides a broad overview of the many facets of diseases due to *Aspergillus* infection and focuses on the role of IgE in allergic bronchopulmonary aspergillosis (ABPA). IgE is involved in the immunopathogenesis of ABPA and is useful in the diagnosis and monitoring of the course of the disease. The future development of diagnostic modalities and innovative therapies targeted at altering IgE response may modulate the course of the disease. The words of Machiavelli summarize the magnitude of this disease: ''In the beginning of the malady it is easy to cure but difficult to detect, but in the course of time, it becomes easy to detect but difficult to cure.''

The protean manifestations of aspergillosis are attributed to colonization, allergy, or tissue invasion by *Aspergillus*. Approximately 350 species of aspergillus have been described, but only a few cause human disease. *Aspergillus fumiga-*

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tus (Af) is most common. *A. niger*, *A. flavus*, and *A. glaucis* cause human disease less frequently, and the other species rarely. A variety of toxins, aflatoxins, clavicin, ochratoxins, and others are produced in vitro by *Aspergillus* species. In vivo production of toxins by *Aspergillus* species has not been demonstrated (2–4).

Aspergillus spores or conidia are 2.5–3.0 μm in diameter and are likely to reach the small airways and alveoli upon inhalation. Once inhaled, the conidia germinate to grow in the hyphal form. The hyphae measure 7–10 μm in size and divide and branch at 45° angle form. *Aspergillus fumigatus* spores are capable of hyphal growth at body temperature (5).

The immune status is the most important determinant of infection. Alveolar macrophages present the first line of host defense to the conidia. The conidia convert to the tissue-invasive hyphal form. Polymorphonuclear cells clear the hyphal forms of *Aspergillus* via an oxidative antifungal mechanism (6). The spectrum of disease in pulmonary aspergillosis is the result of the altered balance between the host defense and fungal growth (3,4,7–10). Impairment of the host defense favors fungal growth.

II. Protean Manifestations of Aspergillosis

The protean manifestations of pulmonary aspergillosis (4) consist of:

1. Saprophytic aspergillosis, consisting of fungal growth without tissue invasion. Examples include airway colonization as in asthma, cystic fibrosis, and COPD; mycetoma; or an invasion of necrotic tissue, usually the infarcted lung.
2. Allergic aspergillosis, a noninfectious inflammatory response to the fungus. Examples include extrinsic allergic alveolitis, eosinophilic pneumonia, and ABPA.
3. Invasive aspergillosis, where there is invasion of viable pulmonary tissue by the fungus. Examples include patients with neoplasia, especially those with acute leukemia; organ transplant; uncontrolled diabetes mellitus; and corticosteroid therapy.

III. Allergic Bronchopulmonary Aspergillosis

ABPA is an immunologically mediated disease and is a result of hypersensitivity to the antigen of the fungus *Aspergillus*, especially *A. fumigatus*. Other species of *Aspergillus* (*A. flavus*, *A. niddus*, *A. niger*, *A. ocharceus*, *A. oryzae*, *A. terreus*) cause the disease infrequently (2,11–14). Other fungi, such as *Candida* (15), *Curvuleria* (16–18), *Fusarium* (19), *Pseudoallescheria* (20), *Helminthosporium* (21), *Penicillium* (22), *Stemphilium* (23), *Torulopsis* (24), *Bipolaris* (18), and

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Dreschlera (25), can cause similar disease. Therefore, the terms allergic bronchopulmonary mycoses (ABPM), allergic bronchopulmonary fungosis (ABPF), allergic bronchopulmonary candidiasis (ABPC), and allergic bronchopulmonary helminthosporosis (ABPH) have been used to describe illnesses similar to ABPA (26,27).

ABPA was first described in 1936 in a young boy with a family history of atopy, who had atopic eczema followed by asthma, eosinophilia, and proximal bronchiectasis (28). Five years later, similar patients with a positive family history were recognized (29). However, it was not until 1952 (30), when the offending organism, *A. fumigatus*, responsible for ABPA was recognized in England. The first case of ABPA in the United States was identified in 1968 (31). Since then, many reports of ABPA have appeared in the literature (5,27,32–34).

ABPA occurs in 1–2% of patients with asthma (10,27) and in up to 10% (7–35%) of patients with cystic fibrosis (10,27,35). It is characterized by intermittent or continuous colonization of the bronchi by the fungus in conjunction with the unique immunological reaction and pathophysiogical findings. The conidia and hyphae have receptors for anti-Af IgE (26,36).

ABPA is an indolent disease with a protracted course, and its effects range from asthma to severe bronchiectasis and fibrosis. It is conjectured that most ABPA begins during childhood, often with asymptomatic pulmonary involvement and exacerbation that make an early diagnosis difficult. The result is that patients often progress to the late fibrotic stage (27,33,37). Acute episodes are not particularly characteristic and may be indistinguishable from an exacerbation of asthma (5,27). Most patients are 20–40 years old at the time of diagnosis. They are usually atopic. Asthma is present in a majority of patients (34) and may precede the development of ABPA. There may be other manifestations of allergic diseases, including rhinitis, urticaria, eczema, conjunctivitis, food allergy, and anaphylaxis (5).

The disease usually manifests with fever, constitutional symptoms, wheezing, productive cough with mucus plugs or flecks, and increasing dyspnea. Pleuritic chest pain and hemoptysis are common (9). Spontaneous pneumothorax (38,39), pleural effusion(s) (40), pulmonary (41) or cerebral (42) aspergilloma, and allergic angitis (43) are uncommon. The examination may reveal adventitious sounds, rhonchi with prolonged expiration, or rales in those with pulmonary infiltrates. Clubbing and cyanosis are late manifestations of the disease.

IV. IgE and Immunopathogenesis

The immunopathogenesis of ABPA is still incompletely understood. The clinical manifestations of disease may result from complex interactions between the host and the fungal organism and the host's genetically determined immune response.

Several host factors, including the humoral response with specific IgE-

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mediated type I and specific IgG-mediated type III hypersensitivity reactions, IgE elaborated to different antigenic proteins (in particular 18 kDa Asp f1 protein), surfactant proteins, cellular immunity and the cytokine response, and HLA-DR subtypes, are believed to be essential in the pathogenesis of disease.

Various host factors play an important role. In the normal host, the mucus-epithelial barrier, mucociliary clearance, and phagocytosis by alveolar macrophages limit antigenic exposure to inhaled fungal spores and antigens. However, in atopic individuals as well as in patients with cystic fibrosis, pathogenesis involves the inhalation, trapping, and subsequent germination of *Aspergillus* spores in the viscid secretions of the airways. This exposure to fungal antigens triggers the formation of IgE antibodies and the inflammatory cascade (Fig. 1). Mast cell degranulation and eosinophilic infiltration occur with fungal antigen reexposure (44,45). Previous studies (44,46–48) have suggested that fungal proteases may play a role in this inflammatory response.

Specific IgE-mediated type I and IgG-mediated type III hypersensitivity reactions are postulated to play a fundamental role in immunopathogenesis. IgA-Af may have a contributory role in the pathogenesis of ABPA (49). Patients with ABPA have a significantly elevated level of serum IgE compared with normals and those with uncomplicated asthma. In patients with ABPA, the unstimulated peripheral blood mononuclear cells elaborate greater levels of IgE than those in normal individuals or in patients with uncomplicated asthma (50). Furthermore, anti-*Candida albicans* IgE and IgG subclasses may participate in the pathophysiology of ABPA by exacerbating pulmonary opacities (IgE) and inducing eosinophil-mediated inflammatory reaction (IgG1, IgG3) (51).

IgE is also elaborated to different antigenic proteins contained in *Aspergillus* extracts. A number of immunologically important antigens and allergens of *A. fumigatus* have been characterized (10,52–57). The dominant protein, 18 kDa Asp f1 protein, a potent ribotoxin, is believed to play a major role in the immunohistopathology of ABPA (58,59). It is toxic to surrounding tissue and incites elaboration of IgE production and further inflammatory activity (58). Asp f2 is also a major allergen associated with ABPA, especially in patients who have characteristic central bronchiectasis (54–56). Evaluation of the IgE-binding epitopes of Asp f2, a major allergen, has revealed that either the N- or C-terminal region of the protein is essential for the correct folding and conformation for IgE antibody binding and may contribute to understanding the immunoregulation and immunodiagnosis (60).

Figure 1 In ABPA, fungal antigens trigger the formation of IgE antibodies and the inflammatory cascade. (a) Healthy individual; (b) individual with ABPA. (Adapted from Ref. 44.)

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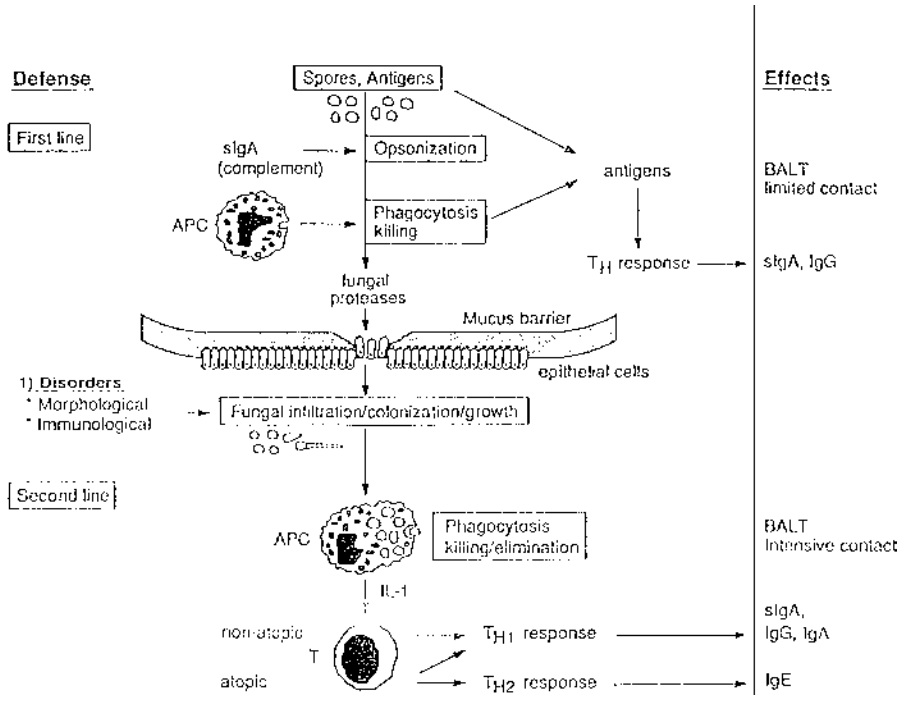

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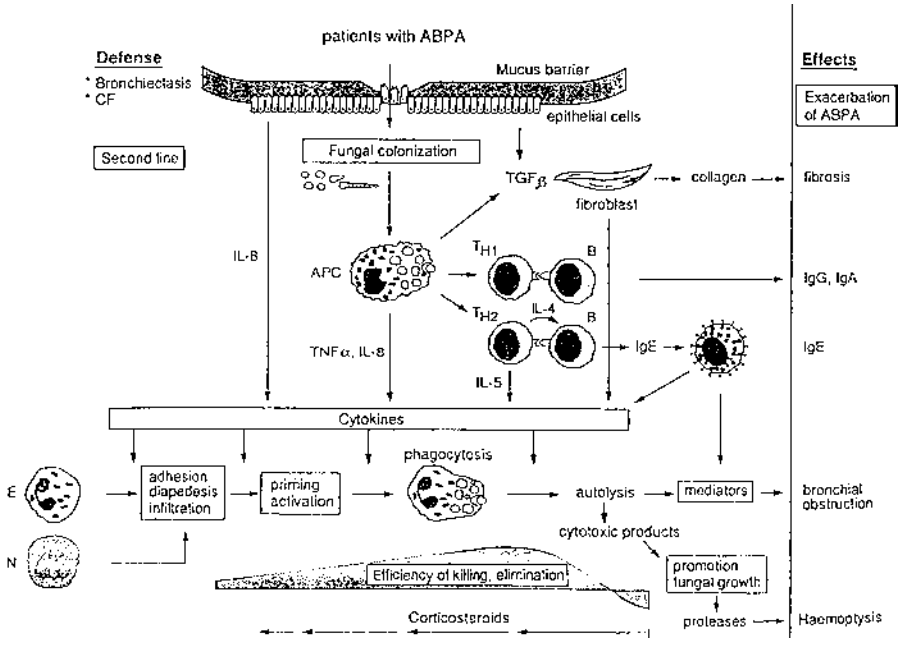

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The host's surfactant proteins may determine the importance of IgE in pathogenesis (61). Surfactant proteins A (SP-A) and D (SP-D) are capable of completely blocking the binding of allergen-specific IgE to *Aspergillus* antigens within the physiological range of concentrations. It is suggested that the ability of SP-A and SP-D to bind certain antigens is mediated through the interaction of their carbohydrate recognition domains with carbohydrate residues on the *Aspergillus* antigens (61). SP-A and SP-D bound to *Aspergillus* antigen significantly reduce the antigen-induced histamine release from sensitized basophils. The SP-A and SP-D proteins may be a protective mechanism against the inhaled antigen in the normal host by blocking IgE binding and thereby reducing histamine release. The mechanisms of up- and downregulation of surfactant protein levels in ABPA may provide additional insight into the pathogenesis of disease (61).

Cell-mediated immunity and cytokine production play a role in the elaboration of IgE and the pathogenesis of ABPA. Bronchoalveolar lavage (BAL) fluid from ABPA patients have revealed significantly increased levels of interleukin-4 (IL-4) and IL-5, with low but significantly elevated IL-2 and interferon-γ (IFN-γ) (62). CD4+ lymphocytes are divided into two subtypes, Th1 and Th2, according to the pattern of cytokine secretion. Th1 secrete IFN-γ and tumor necrosis factor-β (TNFβ), activate macrophages, regulate cell-mediated immunity reactions and cytotoxic lymphocytes, and provide help for IgG2a production. Th2 cells secrete IL-4 and IL-5, provide help for IgE elaboration, and enhance eosinophil production, survival, and activity. The majority of T-cell clones from ABPA patients follow a typical Th2 profile (45). Chauhan et al. (63), in a study of T-cell clones specific for the Asp f1 antigens, found that most were IL-4–producing CD4+ Th2 cells. Cytokines elaborated by a particular subset generally inhibit the proliferation of the other subset (63). This increased antigen-specific Th-2 response was also noted in the subset of ABPA patients with CF (64).

Blocking studies using monoclonal antibodies specific for class I HLA-D region gene products showed that the majority of T cells responded to antigen only in association with specific HLA-DR subtypes (HLA-DR2 or HLA-DR5) (65). A larger study suggested that six identified HLA-DR subtypes are central to the pathophysiology of ABPA (65). This observation may explain the variability in the development of ABPA in atopic patients with asthma and cystic fibrosis.

Murali et al. (50), however, did not find a clear Th2 pattern. Their investigation found that stimulation of peripheral blood mononuclear cells from ABPA patients with two *Asperquillus* antigens, 35 kDa antigen and heat-shock protein HSP 1, increased the production of two Th1 cytokines, IFN-γ and IL-2, and the production of the Th2 cytokine IL-5. IgE production also decreased, probably related to the increase in IFN-γ. Murali et al. (50) found that 35 kDa and HSP 1 antigens from Af stimulates the downregulation of IgE production in vitro and is capable of causing eosinophilia in ABPA due to an increase in IL-5.

Regarding the Th1/Th2 paradigm, Schuyler (66) states that this decrease in

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IgE is discordant with the actual increase in IgE observed in ABPA patients. Criticisms are that peripheral blood mononuclear cells may not accurately reflect the profile of pulmonary monocytes and the ABPA patients were being treated with corticosteroids, which may cause a shift of both airway and peripheral blood T cells to a Th1 profile (45,67). In addition, the *Aspergillus* antigen chosen may not be responsible for in vivo increase in specific IgE, in vitro culture may not be representative of events leading to clinical ABPA, and limitations of experimental protocols are derived from current concepts of the rapidly evolving field of immunology (66).

The IL-10 response is another host factor that may contribute to the pathogenesis of ABPA (45). The bronchial epithelial cells produce IL-10, which in vitro inhibits alveolar macrophage and dendritic cell cytokine production (68,69). Pleomorphisms in the human IL-10 gene have been demonstrated (70). In the laboratory, IL-10 gene knockout mice have a 50–60% mortality rate in response to repeated *A. fumigatus* inhalation compared with the wild-type rate of less than 15% (71). In response to antigen, the IL-10 knockout mice showed increased airway inflammation and levels of IL-5 and IF-γ (45,71). IL-10 can suppress the inflammatory response. However, the degree of suppression depends on the genetic background and the route of sensitization (45,71).

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene have been described in ABPA patients without clinical evidence of cystic fibrosis. Gene mutations may play an etiologic role in a subset of the patients with ABPA (72).

V. Pathology

The histological features have not been extensively investigated, as the diagnosis is established on the basis of clinical, radiographic, and laboratory findings (73). Bronchocentric granulomatosis (BCG), eosinophilic pneumonia, and mucoid impaction of bronchi (MIB) have been described in cases of ABPA (73). Bosken et al. (74) studied the histological features in lung biopsy or resection specimens of patients with ABPA. The bronchi and bronchioles were most frequently involved. BCG, mucoid impaction, and exudative bronchiolitis were present. The exudative bronchiolitis is characterized by filling of bronchiolar lumens with necrotic neutrophils, eosinophils, and cellular debris within a mucinous or proteinaceous background and occurs distal to areas of BCG (73,74). A chronic cellular inflammatory infiltrate with numerous plasma cells and eosinophils surround the bronchioles, tissue eosinophilia in all cases, and focal eosinophilic pneumonia in over two thirds of cases were noted (73,74). Fungal hyphae were found in three fourths of cases and were located within the centers of BCG and intraluminal mucin in MIB. However, no evidence of direct fungal invasion into viable tissue or blood vessels was present. The presence of BCG or MIB or both with

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tissue eosinophilia is highly suggestive of ABPA or a related fungal hypersensitivity reaction. These findings can be considered diagnostic if fungal hyphae are also present (73).

VI. Diagnosis

A. Diagnostic Criteria

Early diagnosis and treatment is essential to prevent irreversible pulmonary damage. Clinical, radiographic, and laboratory findings help establish the diagnosis of ABPA. The diagnosis can be made with reasonable certainty if six of the eight primary criteria are present (10,73,75,76). These criteria are episodic asthma, immediate skin reactivity to Af antigen, peripheral eosinophilia, transient or fixed pulmonary infiltrates, central bronchiectasis, elevated serum IgE (>1000 ng/mL), presence of precipitating antibodies to *A. fumigatus* (IgG and IgM), and elevated serum specific IgE and IgG antibodies to *A. fumigatus*. The acronym ARTEPICS has been coined by Zhaoming and Lockey (27) for the diagnostic criteria of ABPA: *a*sthma, *r*adiographic pulmonary infiltrates, *t*est of Af positive in skin, *e*osinophilia, *p*recipitating antibodies to *A. fumigatus*, elevated *I*gE in serum, *c*entral bronchiectasis, *s*erum-specific IgE Af and IgG Af elevated.

The major criteria may not all be met. Schonheyder et al. (77) have suggested the concept of ''silent'' ABPA, where ongoing damage to the respiratory mucosa occurs in response to exposure to *Aspergillus conidia* but the diagnostic criteria are not met.

Secondary criteria (10,27), such as *A. fumigatus* in sputum by repeated cultures or microscopic examination, expectoration of brown mucus plugs or flecks containing eosinophils and Charcot-Leyden crystals, and late skin reactivity to *Aspergillus* antigen, are less frequently seen and less important.

B. Serology, Antigens, and Laboratory Diagnosis

It is essential to differentiate ABPA from allergy in patients with asthma or cystic fibrosis. ABPA is a very difficult syndrome to diagnose. Levels of total IgE and specific IgE for aspergillus (IgE-Af) are increased (9,26,78–81). The clinical and laboratory features may be rarely present in the absence of increased IgE. Although elevated *Aspergillus*-specific IgE levels are the hallmark of ABPA, the disease has been described in a child with CF and reduced IgE levels and also in an adult with common variable hypogammaglobulinemia (82,83). Serum precipitating antibodies or precipitins (IgG) are positive in >90% of patients with consistent clinical and radiographic findings, but the diagnosis should not be excluded if the precipitating antibody to *aspergillus* (IgG-Af) is absent despite compatible clinical and radiographic findings (9,84).

An index of IgE is useful to distinguish the patient with ABPA from asthma

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and a positive skin test to *Aspergillus* (85,86). The radioimmunoassay is used to determine the indices whereby sera from ABPA and asthma patients are serially diluted, and the levels of each antibody are compared.

A major problem in the diagnosis of ABPA is the lack of standardized fungal extracts (57). There are several qualitative and quantitative differences in the antigenic extracts prepared in various laboratories and the conditions of cultures (87). The incubation period, the conditions under which the cultures are held, and the composition of the culture medium are critical reasons for the production of different antigenic patterns (10). The form of the fungus from which the antigenic mixture is extracted, the method of extraction, and the subcellular source of antigens also affect antigenic composition. Furthermore, the antigens expressed in vivo during colonization of host tissues are different from those expressed in vitro.

Several investigators (54–58,88–92) are pursuing the use of standardized, recombinant antigens and PCR-DNA, which may improve the ability to diagnose ABPA. Peptides at 46–65 and 106–125 on the Asp f1 protein elicit dominant reactions, and specific antibodies to these sites may be a marker for patients with ABPA (58,93). Use of gp66, formerly antigen 7, improved specific IgE detection for the diagnosis of ABPA (88). Crameri et al. (89) found that the dissection of the IgE-mediated immune response to single recombinant *A. fumigatus* in asthmatic patients allows the discrimination between ABPA and Af sensitization with high specificity and sensitivity. Nonsecreted manganese superoxide dismutase recombinant *Aspergillus* (rAsp f6) and the rAsp f4 allergen are exclusively recognized by IgE of ABPA patients (89). Hemmann et al. (90) demonstrated differential IgE responses to allergens in the Af-sensitive CF patients with or without ABPA and controls. Intracellular manganese superoxide dismutase (rAsp f6) and rAsp f4 are recognized exclusively by IgE from sera of CF patients with ABPA. Asp f4 and Asp f6 represented specific markers for ABPA and allow a sensitive and fully specific diagnosis of the disease process (90).

In addition, Hemmann et al. (91) studied the recombinant Asp f3 (rAsp f3) based serological determinations in ELISA and ImmunoCAP and compared to skin test response in Af-sensitive subjects. Recombinant Asp f3 represents a major allergen of Af. It is recognized by 84% of asthmatic individuals sensitized to fungus and elicits specific type I skin reactions. The analysis of rAsp f3-specific immunoglobulins showed significantly higher serum levels of IgG, IgG1, IgG4, and IgE antibodies in ABPA patients, compared with Af-sensitized asthmatics and healthy controls. The skin test outcome correlated with the presence of rAsp f3-specific IgE and suggested that serological data with recombinant allergens may be used to diagnose sensitization (91).

The 37 kDa protein with complete N-terminal homology to Asp f2 is a major allergen of Af that significantly reacts with IgE antibody in patients with ABPA but not in *Aspergillus*-sensitive subjects with asthma or in normal controls

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(54,56,60). Asp f2, a major allergen of Af, exhibited IgE antibody binding with sera from patients with ABPA, suggesting its potential role in the diagnosis of the disease (56,60).

PCR *Aspergillus* DNA in serum has been used for the detection of *A. fumigatus* antigen in sera for the diagnosis of pulmonary aspergillosis and appears to have the highest sensitivity (92).

C. Skin Test Reactivity

An immediate skin test reactivity to Af antigen (IgE dependent) is essential to make the diagnosis. A late reaction (Arthus reaction) is positive only in some patients (9). Dual reaction (immediate and late) occurs in up to 33% of cases (34).

D. Eosinophils

Peripheral blood eosinophilia is often seen with absolute counts over 500 cells · mm^{-3} and usually over 1000 cells · mm^{-3} in the range of 1000–1500 cells · mm^{-3}. ABPA has been identified in patients without elevated eosinophil counts (9). Peripheral eosinophil counts of greater than 30–40% makes the diagnosis of ABPA unlikely (9,32).

E. Chest Roentgenograph and Computed Tomography

A wide variety of roentgenographic changes has been reported in patients with ABPA (3,27,94,95) Nonspecific radiographic changes include normal chest x-ray, hyperinflation, various patterns of migratory opacities, consolidation, atelectasis, and nodules. Pleural thickening and pseudo-hilar and hilar (96) adenopathy may occur. Parallel lines with ring shadows, ''gloved finger,'' ''toothpaste'' shadows, and band and tramline shadows indicate bronchial involvement. Specific changes include central or proximal bronchiectasis without obliteration of distal bronchi and are characteristic of the disease. These changes are more marked in the upper lobes than in the lower lobes. Pulmonary fibrotic changes and resultant shrinkage occurs exclusively in upper lung zones and is seen in the advanced stages of the lung disease (Fig. 2).

The severity of radiological changes correlate with the immunological findings. Patients without central bronchiectasis have lower concentrations of total serum IgE, serum anti-Af-IgE, anti-Af-IgA, and anti-Af-IgG in comparison to those with ABPA and central bronchiectasis (97). Kiely et al. (94) devised a six-stage radiographic scoring system (score 0–5) based on the severity and duration of the radiographic changes seen: 0: normal; 1: transient hyperinflation; 2: transient minor changes; 3: transient major changes; 4: permanent (>6 months duration) minor changes; and 5: permanent major changes. A positive correlation was observed between the severity of chest radiographic findings and the periph-

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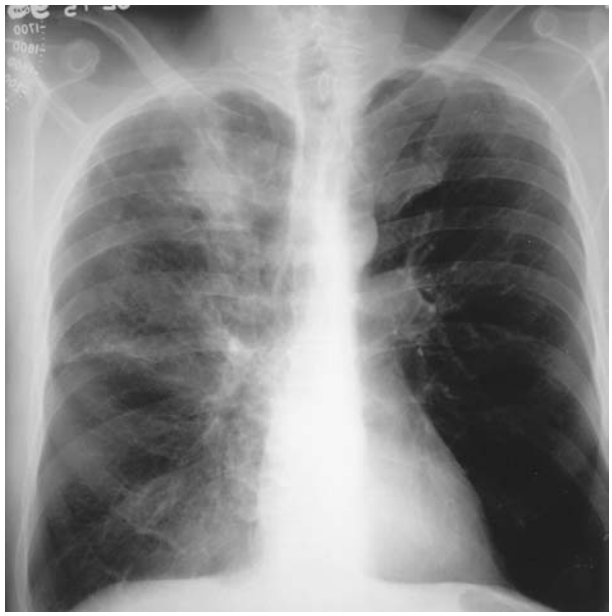

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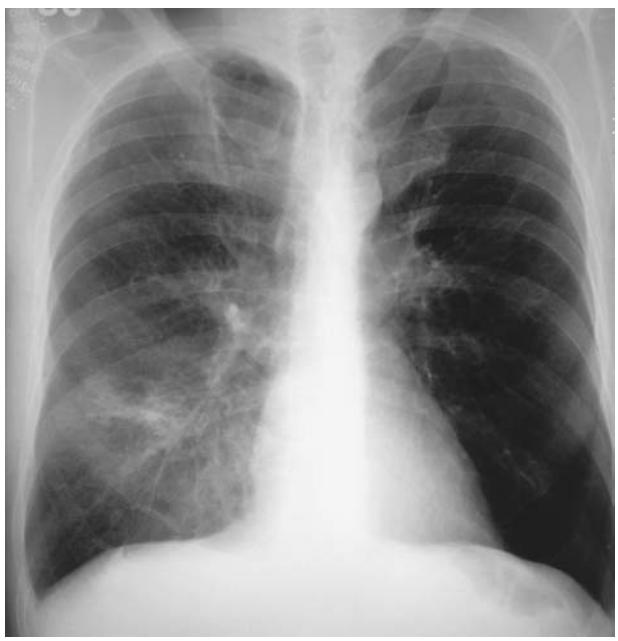

(b)

Figure 2 Serial chest radiographs of a patient with ABPA showing the waxing and waning (a,b), recurrence (c), and resolution (d,e) of opacities. The CT of the same patient reveals bronchiectasis (f).

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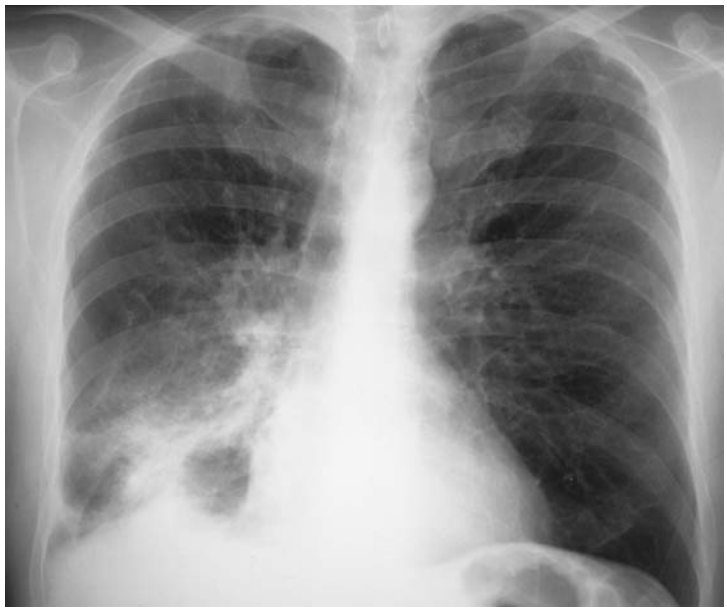

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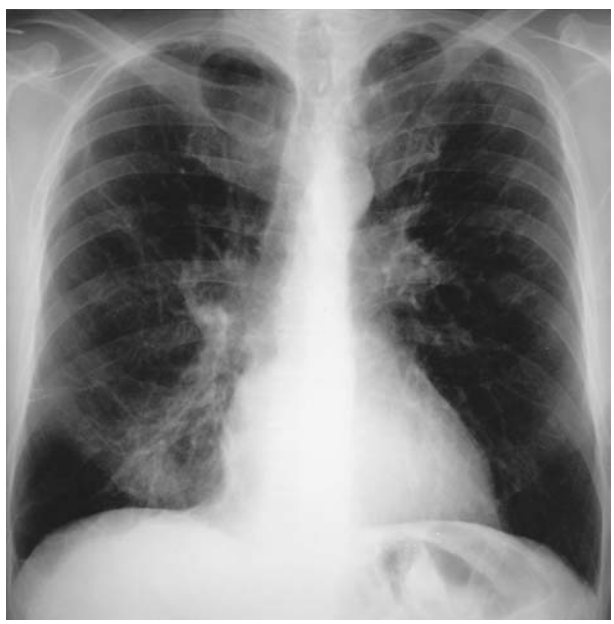

(d)

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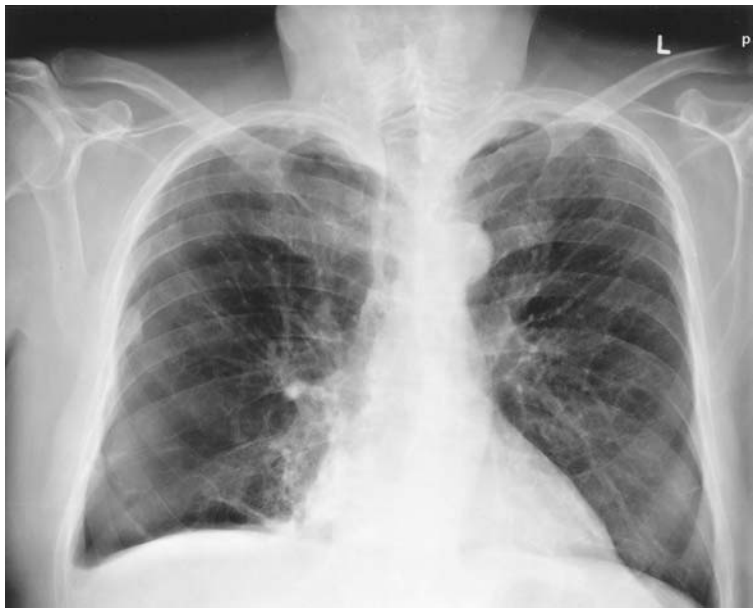

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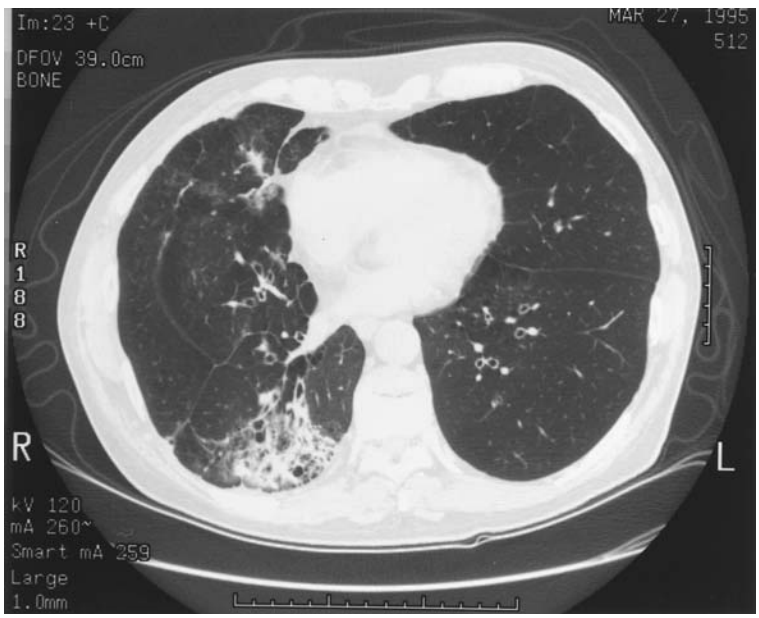

(f)

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eral eosinophil count and Af index. However, total serum IgE did not correlate with chest radiographic staging in their study (94).

Computed tomography (CT) is helpful for demonstrating bronchiectasis and monitoring the stage of the disease (Fig. 2f). Characteristic CT appearances are those of central bronchiectasis, mucus plugging, appearing as branching structures, more common in the upper lobes. The mucus plugs may have high attenuation on CT. Occasionally air-fluid levels, aspergilloma in chronic ABPA, peripheral mass lesions mimicking eosinophilic pneumonia, and pleural thickening may be seen (98–102).

F. Pulmonary Function

Pulmonary function test (PFT) abnormalities include varying degrees of partial or completely reversible airflow limitation. An irreversible restrictive or mixed restrictive and obstructive pattern occurs in the late stages of the disease (27).

G. Bronchoscopy

Elevated levels of IgE-Af are found in BAL fluid reflecting the role of the lung as a specific immunological organ (9). Flexible fiberoptic bronchoscopy with bronchial washing and bronchial biopsy is reportedly useful in patients suspected of ABPA, where radiographic and immunological findings are not characteristic of the disease mucus plug(s) containing eosinophils intermixed with Charcot-Leyden crystals, scattered fungal hyphae, and allergic mucin, i.e., abundant eosinophils arranged in parallel layers on biopsy specimen are diagnostic of ABPA (10,103,104).

VII. Clinical Stages

Based on the clinical, radiographic, and laboratory (peripheral eosinophils and immunological) findings, ABPA is divided into five stages (9,26,33) to aid in therapeutic and prognostic response to treatment (Table 1). These stages do not represent the phases of the disease, nor is there a relentless progression of the disease from stage I to stage V. Stage I, acute ABPA, fulfills the diagnostic criteria with chest roentenographic opacities usually in the middle or upper lobes, peripheral eosinopilia, markedly elevated total serum IgE, and sputum with Af and eosinophils. Stage II, remission, occurs with a decrease in opacities and IgE. In stage III, exacerbation, the previous criteria of stage I reappears. In stage IV ABPA, corticosteroid-dependent asthma, the patient is unable to be tapered off of steroid therapy. The total IgE may remain elevated. After prolonged therapy with prednisone for asthma and control of ABPA, the IgE and IgG antibody indices against *A. fumigatus* may remain elevated or may be below the levels that are of diagnostic. Stage V, the fibrotic state, is characterized by irreversible lung disease (33,80,105).

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Table 1 Allergic Bronchopulmonary Aspergillosis: Clinical Stages

Stage	Clinical features	Chest radiograph	Serum IgE		Peripheral eosinophilia	Precipitating antibody to Af
			Total	IgE-Af		
I: Acute	Cough, wheezing, fever, sputum plugs may be present	Transient lung infiltrates	+++	+	+	+
II: Remission	Usually no symptoms	No radiographic infiltrate	+	+/−	−	+/−
III: Exacerbation	Fever, cough, and wheezing may be absent	Pulmonary infiltrates	+++	+	+	+
IV: Corticosteroid-dependent asthma	Persistent disabling wheezing	Pulmonary infiltrate usually absent	++	+/−	+/−	+/−
V: Fibrotic	Dyspnea, wheezing, clubbing of the fingers, cyanosis, chronic sputum production	Extensive fibrosis/honeycombing, segmental/lobar atelectasis	+	+/−	−	+/−

IgE: immunoglobulin E; Af: *Aspergillus fumigatus*; IgE-Af: specific IgE direct against Af; +: presence of; −: absence of.
Source: Ref. 9.

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VIII. Treatment

The mainstay of therapy for ABPA is oral corticosteroids. The objectives and endpoint of therapy are to suppress the inflammatory and immunological response in order to preserve lung function and prevent disease progression (26,45,106). Corticosteroids appear to decrease IgE levels and peripheral eosinophilia, clear pulmonary opacities, and decrease bronchospasm (44,45,106).

Serum IgE levels help to identify flares, although total serum IgE will not return to normal. Levels should be correlated with the clinical course. Steroids should not be used to treat an elevated serum IgE level if the patient appears to be clinically doing well (26,45,105–107). Immunoblot analysis can be used to monitor disease activity and response to treatment. Only in the acute stage of ABPA were IgA antibodies against Af antigens demonstrated, which may be more informative than specific IgG (108).

Exacerbations of underlying asthma may occur. The difficulty is in differentiating episodes of underlying asthma from that of flares of ABPA. In this scenario, an episode of exacerbation of asthma without a flare of ABPA will not have associated radiographic pulmonary opacities and marked increases in IgE, whereas a flare of ABPA may be associated with pulmonary opacities and marked increases in serum IgE. A flare of ABPA may occur with tapering of corticosteroid therapy.

The suggested management plan (26,45,106) is:

1. Prednisone 0.5 mg/kg a day, single dose therapy, over 2 weeks.
2. Prednisone 0.5 mg/kg QOD given for 3 months, then tapered and discontinued during the next 3 months.
3. After the initial clearing, a chest radiograph is obtained every 4 months over the next 2 years, then every 6 months for 2 years, then annually.
4. IgE levels are the best indicator of disease activity. IgE level should be checked monthly and should decrease in 1–2 months and plateau after 6 months. A two- to threefold rise in total IgE suggests recrudescence. An increase in IgE level is often followed by an ABPA flare. If 2 years elapse without recurrence, then total serum IgE should be measured every month.
5. A chest radiograph should be obtained. If an opacity is present, the patient should be treated with corticosteroids.
6. PFT should be obtained annually.

ABPA may flare after a prolonged remission. Steroid-dependent asthma symptoms may occur. Untreated ABPA may lead to end-stage pulmonary fibrosis and bronchiectasis.

Itraconazole, an orally active triazole antifungal agent with activity against *Aspergillus*, is effective in treating *Aspergillus* infection. It may be an adjunct

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to corticosteroid therapy in difficult-to-control patients or patients requiring high doses of steroids. Denning et al. (109), in a small series of ABPA patients treated with itraconazole, noted improvement in PFT, a decrease in eosinophil count and IgE, and suggested a possible steroid sparing effect. A retrospective review of a larger series of patients with ABPA in CF revealed that itraconazole treatment is safe and associated with fewer acute episodes of ABPA despite reduction in oral steroid dose (110).

Lung transplantation may be necessary in patients with ABPA. Recurrence of ABPA has been reported in a patient with CF in the posttransplant lung (111). The future development of novel therapeutic approaches targeted at altering IgE response may modulate the course of the disease. Investigation into the identification of the IgE-downregulating epitopes in Af antigens may potentially be of therapeutic significance (50). Cell-mediated immunity and cytokine production may have important implications for therapy. Th1 associated cytokines such as IF-γ may be an effective treatment in ABPA (63). The clinical efficacy of endogenous protease inhibitors for prophylaxis and treatment remains to be elucidated (112). The role of anti-IgE monoclonal antibody therapy (113–116) in patients with ABPA remains to be determined.

IX. Conclusions

ABPA affects asthma and cystic fibrosis patients. It is probably a genetically determined inflammatory and immune-mediated host response to chronic colonization of the airways with *Aspergillus*. IgE has a fundamental role in the immunopathogenesis of ABPA and as a marker of disease. ABPA consists of a spectrum of mild to severe manifestations ranging from asthma to fibrosis and bronchiectasis. Patients will have recurrent wheezing, mucus production, pulmonary opacities, and elevated IgE and may progress to the development of central bronchiectasis and end-stage fibrotic lung disease. The disease may be difficult to diagnose, is readily responsive to therapy in the early stages, but in the course of time and in the later stages, it is difficult to cure. Future investigations will facilitate the early diagnosis and develop innovative therapies, which may prevent progression to the later stages of disease.

References

1. Shah A. Allergic bronchopulmonary aspergillosis. Indian J Chest Dis Allied Sci 1998; 40:41–54.
2. Andriole VT. Infections with *Aspergillus* species. Clin Infect Dis 1993; 17(suppl 2):S481–S486.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

3. Gefter WB. The spectrum of pulmonary aspergillosis. J Thorac Imaging 1992; 7: 56–74.
4. Fraser RS. Pulmonary aspergillosis: pathologic and pathogenetic features. Pathol Annu 1993; 28:231–277.
5. Elliott MW, Newman Taylor AJ. Allergic bronchopulmonary aspergillosis. Clin Exp Allergy 1997; 27(suppl 1):55–59.
6. Waldorf AR. Pulmonary defense mechanisms against opportunistic fungal pathogens. Immunol Ser 1989; 47:243–271.
7. Broderick LS, Conces DJ, Jr, Tarver RD, Bergmann CA, Bisesi MA. Pulmonary aspergillosis: a spectrum of disease. Crit Rev Diagn Imaging 1996; 37:491–531.
8. Miller WT. Aspergillosis: a disease with many faces. Semin Roentgenol 1996; 31: 52–66.
9. Sharma OP, Chwogule R. Many faces of pulmonary aspergillosis. Eur Respir J 1998; 12:705–715.
10. Latge JP. *Aspergillus fumigatus* and aspergillosis. Clin Microbiol Rev 1999; 12: 310–350.
11. Novey HS, Wells ID. Allergic bronchopulmonary aspergillosis caused by *Aspergillus ochraceus*. Am J Clin Pathol 1978; 70:840–843.
12. Akiyama K, Takizawa H, Suzuki M, Miyachi S, Ichinohe M, Yanagihara Y. Allergic bronchopulmonary aspergillosis due to *Aspergillus oryzae*. Chest 1987; 91: 285–286.
13. Laham MN, Carpenter JL. *Aspergillus terreus*, a pathogen capable of causing infective endocarditis, pulmonary mycetoma, and allergic bronchopulmonary aspergillosis. Am Rev Respir Dis 1982; 125:769–772.
14. Nakahara Y, Katoh O, Yamada H, Sumida I, Hanada M. Allergic bronchopulmonary aspergillosis caused by *Aspergillus terreus* presenting lobar collapse. Intern Med 1992; 31:140–142.
15. Akiyama K, Mathison DA, Riker JB, Greenberger PA, Patterson R. Allergic bronchopulmonary candidiasis. Chest 1984; 85:699–701.
16. McAleer R, Kroenert DB, Elder JL, Froudist JH. Allergic bronchopulmonary disease caused by *Curvularia lunata* and *Drechslera hawaiiensis*. Thorax 1981; 36: 338–344.
17. Halwig JM, Brueske DA, Greenberger PA, Dreisin RB, Sommers HM. Allergic bronchopulmonary curvulariosis. Am Rev Respir Dis 1985; 132:186–188.
18. Lake FR, Froudist JH, McAleer R, Gillon RL, Tribe AE, Thompson PJ. Allergic bronchopulmonary fungal disease caused by Bipolaris and Curvularia. Aust NZ J Med 1991; 21:871–874.
19. Backman KS, Roberts M, Patterson R. Allergic bronchopulmonary mycosis caused by *Fusarium vasinfectum.* Am J Respir Crit Care Med 1995; 152:1379–1381.
20. Miller MA, Greenberger PA, American R, Toogood JH, Noskin GA, Roberts M, Patterson R. Allergic bronchopulmonary mycosis caused by *Pseudallescheria boydii*. Am Rev Respir Dis 1993; 148:810–812.
21. Halloran TJ. Allergic bronchopulmonary helminthosporiosis [letter]. Am Rev Respir Dis 1983; 128:578.
22. Fergusson RJ, Milne LJ, Crompton GK. Penicillium allergic alveolitis: faulty installation of central heating. Thorax 1984; 39:294–298.

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23. Benatar SR, Allan B, Hewitson RP, Don PA. Allergic bronchopulmonary stemphyliosis. Thorax 1980; 35:515–518.
24. Patterson R, Samuels BS, Phair JJ, Roberts M. Bronchopulmonary torulopsosis. Int Arch Allergy Appl Immunol 1982; 69:30–33.
25. Matthiesson AM. Allergic bronchopulmonary disease caused by fungi other than *Aspergillus* [letter]. Thorax 1981; 36:719.
26. Greenberger PA. Diagnosis and management of allergic bronchopulmonary aspergillosis. Allergy Proc 1994; 15:335–339.
27. Zhaoming W, Lockey RF. A review of allergic bronchopulmonary aspergillosis. J Investig Allergol Clin Immunol 1996; 6:144–151.
28. Chobot R. Bronchiectasis as a complicating cause of asthma. Am J Dis Child 1936; 52:882.
29. Ordstrand HSV. Bronchiectasis. In: Thomas JW, ed. Allergy in Clinical Practice. Philadelphia: J.B. Lippincott Company, 1941:93–111.
30. Hinson KFW, Moon AJ, Plummer NS. Bronchopulmonary aspergillosis. Thorax 1952; 7:317–333.
31. Patterson R, Gilbert TM. Hypersensitivity disease of the lung. U Mich Med 1968; 34:8–11.
32. Greenberger PA, Patterson R. Diagnosis and management of allergic bronchopulmonary aspergillosis. Ann Allergy 1986; 56:444–448.
33. Patterson R, Greenberger PA, Radin RC, Roberts M. Allergic bronchopulmonary aspergillosis: staging as an aid to management. Ann Intern Med 1982; 96:286–291.
34. McCarthy DS, Pepys J. Allergic bronchopulmonary aspergillosis. Clinical immunology. 2. Skin, nasal and bronchial tests. Clin Allergy 1971; 1:415–432.
35. Becker JW, Burke W, McDonald G, Greenberger PA, Henderson WR, Aitken ML. Prevalence of allergic bronchopulmonary aspergillosis and atopy in adult patients with cystic fibrosis. Chest 1996; 109:1536–1540.
36. Reijula KE, Kurup VP, Fink JN. Ultrastructural demonstration of specific IgG and IgE antibodies binding to *Aspergillus fumigatus* from patients with aspergillosis. J Allergy Clin Immunol 1991; 87:683–688.
37. Greenberger PA, Patterson R, Ghory A, Arkins JA, Walsh T, Graves T, Saker J. Late sequelae of allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 1980; 66:327–335.
38. Ricketti AJ, Greenberger PA, Glassroth J. Spontaneous pneumothorax in allergic bronchopulmonary aspergillosis. Arch Intern Med 1984; 144:151–152.
39. Judson MA, Marshall C, Beale G, Holt JB. Pneumothorax and bronchopleural fistula during treatment of allergic bronchopulmonary aspergillosis. South Med J 1993; 86:1061–1063.
40. Murphy D, Lane DJ. Pleural effusion in allergic bronchopulmonary aspergillosis: two case reports. Br J Dis Chest 1981; 75:91–95.
41. Anderson CJ, Craig S, Bardana EJ, Jr. Allergic bronchopulmonary aspergillosis and bilateral fungal balls terminating in disseminated aspergillosis. J Allergy Clin Immunol 1980; 65:140–144.
42. Starke ID, Keal EE. Cerebral aspergilloma in a patient with allergic bronchopulmonary aspergillosis. Br J Dis Chest 1980; 74:301–305.
43. Stephens M, Reynolds S, Gibbs AR, Davies B. Allergic bronchopulmonary asper-

MARCEL DEKKER, INC.
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gillosis progressing to allergic granulomatosis and angiitis (Churg-Strauss syndrome). Am Rev Respir Dis 1988; 137:1226–1228.
44. Kauffman HF, Tomee JF, van der Werf TS, de Monchy JG, Koeter GK. Review of fungus-induced asthmatic reactions. Am J Respir Crit Care Med 1995; 151: 2109–2115; discussion 2116.
45. Cockrill BA, Hales CA. Allergic bronchopulmonary aspergillosis. Annu Rev Med 1999; 50:303–316.
46. Tomee JF, Kauffman HF, Klimp AH, de Monchy JG, Koeter GH, Dubois AE. Immunologic significance of a collagen-derived culture filtrate containing proteolytic activity in *Aspergillus*-related diseases. J Allergy Clin Immunol 1994; 93: 768–778.
47. Tomee JF, Hiemstra PS, Heinzel-Wieland R, Kauffman HF. Antileukoprotease: an endogenous protein in the innate mucosal defense against fungi. J Infect Dis 1997; 176:740–747.
48. Tomee JF, Wierenga AT, Hiemstra PS, Kauffman HK. Proteases from *Aspergillus fumigatus* induce release of proinflammatory cytokines and cell detachment in airway epithelial cell lines. J Infect Dis 1997; 176:300–303.
49. Apter AJ, Greenberger PA, Liotta JL, Roberts M. Fluctuations of serum IgA and its subclasses in allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 1989; 84:367–372.
50. Murali PS, Kurup VP, Bansal NK, Fink JN, Greenberger PA. IgE downregulation and cytokine induction by *Aspergillus* antigens in human allergic bronchopulmonary aspergillosis [see comments]. J Lab Clin Med 1998; 131:228–235.
51. Roig E, Malo JL, Montplaisir S. Anti-*Candida albicans* IgE and IgG subclasses in sera of patients with allergic bronchopulmonary aspergillosis (ABPA). Allergy 1997; 52:394–403.
52. Longbottom JL, Harvey C, Taylor ML, Austwick PK, Fitzharris P, Walker CA. Characterization of immunologically important antigens and allergens of *Aspergillus fumigatus*. Int Arch Allergy Appl Immunol 1989; 88:185–186.
53. Banerjee B, Chetty A, Joshi AP, Puranam US. Identification and partial characterization of diagnostically relevant antigens of *Aspergillus fumigatus*. Asian Pac J Allergy Immunol 1990; 8:13–18.
54. Banerjee B, Kurup VP, Phadnis S, Greenberger PA, Fink JN. Molecular cloning and expression of a recombinant *Aspergillus fumigatus* protein Asp f II with significant immunoglobulin E reactivity in allergic bronchopulmonary aspergillosis. J Lab Clin Med 1996; 127:253–262.
55. Banerjee B, Kurup VP, Greenberger PA, Hoffman DR, Nair DS, Fink JN. Purification of a major allergen, Asp f2 binding to IgE in allergic bronchopulmonary aspergillosis, from culture filtrate of *Aspergillus fumigatus.* J Allergy Clin Immunol 1997; 99:821–827.
56. Banerjee B, Greenberger PA, Fink JN, Kurup VP. Immunological characterization of Asp f2, a major allergen from *Aspergillus fumigatus* associated with allergic bronchopulmonary aspergillosis. Infect Immun 1998; 66:5175–5182.
57. Crameri R. Recombinant *Aspergillus fumigatus* allergens: from the nucleotide sequences to clinical applications. Int Arch Allergy Immunol 1998; 115:99– 114.
58. Knutsen AP, Mueller KR, Hutcheson PS, Slavin RG. Serum anti-*Aspergillus fumi-*

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gatus antibodies by immunoblot and ELISA in cystic fibrosis with allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 1994; 93:926–931.
59. el-Dahr JM, Fink R, Selden R, Arruda LK, Platts-Mills TA, Heymann PW. Development of immune responses to *Aspergillus* at an early age in children with cystic fibrosis. Am J Respir Crit Care Med 1994; 150:1513–1518.
60. Banerjee B, Greenberger PA, Fink JN, Kurup VP. Conformational and linear B-cell epitopes of Asp f2, a major allergen of *Aspergillus fumigatus*, bind differently to immunoglobulin E antibody in the sera of allergic bronchopulmonary aspergillosis patients. Infect Immun 1999; 67:2284–2291.
61. Madan T, Kishore U, Shah A, Eggleton P, Strong P, Wang JY, Aggrawal SS, Sarma PU, Reid KB. Lung surfactant proteins A and D can inhibit specific IgE binding to the allergens of *Aspergillus fumigatus* and block allergen-induced histamine release from human basophils. Clin Exp Immunol 1997; 110:241–249.
62. Walker C, Bauer W, Braun RK, Menz G, Braun P, Schwarz F, Hansel TT, Villiger B. Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. Am J Respir Crit Care Med 1994; 150:1038–1048.
63. Chauhan B, Knutsen A, Hutcheson PS, Slavin RG, Bellone CJ. T cell subsets, epitope mapping, and HLA-restriction in patients with allergic bronchopulmonary aspergillosis. J Clin Invest 1996; 97:2324–2331.
64. Skov M, Poulsen LK, Koch C. Increased antigen-specific Th-2 response in allergic bronchopulmonary aspergillosis (ABPA) in patients with cystic fibrosis [see comments]. Pediatr Pulmonol 1999; 27:74–79.
65. Chauhan B, Santiago L, Kirschmann DA, Hauptfeld V, Knutsen AP, Hutcheson PS, Woulfe SL, Slavin RG, Schwartz HJ, Bellone CJ. The association of HLA-DR alleles and T cell activation with allergic bronchopulmonary aspergillosis. J Immunol 1997; 159:4072–4076.
66. Schuyler M. The Th1/Th2 paradigm in allergic bronchopulmonary aspergillosis [editorial; comment]. J Lab Clin Med 1998; 131:194–196.
67. Krouwels FH, van der Heijden JF, Lutter R, van Neerven RJ, Jansen HM, Out TA. Glucocorticosteroids affect functions of airway- and blood-derived human T-cell clones, favoring the Th1 profile through two mechanisms. Am J Respir Cell Mol Biol 1996; 14:388–397.
68. Bonfield TL, Konstan MW, Burfeind P, Panuska JR, Hilliard JB, Berger M. Normal bronchial epithelial cells constitutively produce the anti-inflammatory cytokine interleukin-10, which is downregulated in cystic fibrosis. Am J Respir Cell Mol Biol 1995; 13:257–261.
69. Nicod LP, el Habre F, Dayer JM, Boehringer N. Interleukin-10 decreases tumor necrosis factor alpha and beta in alloreactions induced by human lung dendritic cells and macrophages. Am J Respir Cell Mol Biol 1995; 13:83–90.
70. Mehrian R, Quismorio FP, Jr., Strassmann G, Stimmler MM, Horwitz DA, Kitridou RC, Gauderman WJ, Morrison J, Brautbar C, Jacob CO. Synergistic effect between IL-10 and bcl-2 genotypes in determining susceptibility to systemic lupus erythematosus. Arthritis Rheum 1998; 41:596–602.
71. Grunig G, Corry DB, Leach MW, Seymour BW, Kurup VP, Rennick DM. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine

MARCEL DEKKER, INC.
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model of allergic bronchopulmonary aspergillosis. J Exp Med 1997; 185:1089–1099.
72. Miller PW, Hamosh A, Macek M, Jr., Greenberger PA, MacLean J, Walden SM, Slavin RG, Cutting GR. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. Am J Hum Genet 1996; 59:45–51.
73. Katzenstein AA. Katzenstein and Askin's surgical pathology of non-neoplastic lung disease. Major Probl Pathol 1997; 13.
74. Bosken CH, Myers JL, Greenberger PA, Katzenstein AL. Pathologic features of allergic bronchopulmonary aspergillosis. Am J Surg Pathol 1988; 12:216–222.
75. Rosenberg M, Patterson R, Mintzer R, Cooper BJ, Roberts M, Harris KE. Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. Ann Intern Med 1977; 86:405–414.
76. Detjen PF, Greenberger PA, Patterson R. Allergic bronchopulmonary aspergillosis. In: Lynch JPI, DeRemee RA, eds. Immunologically Mediated Pulmonary Diseases. Philadelphia: Lippincott, 1996; 378–398.
77. Schonheyder H. Pathogenetic and serological aspects of pulmonary aspergillosis. Scand J Infect Dis Suppl 1987; 51:1–62.
78. Patterson R, Greenberger PA, Halwig JM, Liotta JL, Roberts M. Allergic bronchopulmonary aspergillosis. Natural history and classification of early disease by serologic and roentgenographic studies. Arch Intern Med 1986; 146:916–918.
79. Greenberger PA, Patterson R. Allergic bronchopulmonary aspergillosis. Model of bronchopulmonary disease with defined serologic, radiologic, pathologic and clinical findings from asthma to fatal destructive lung disease. Chest 1987; 91:165S–171S.
80. Leser C, Kauffman HF, Virchow C, Sr., Menz G. Specific serum immunopatterns in clinical phases of allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 1992; 90:589–599.
81. Greenberger PA. Immunologic aspects of lung diseases and cystic fibrosis. JAMA 1997; 278:1924–1930.
82. Maiz L, Cuevas M, Quirce S, Pacheco A, Escobar H. Allergic bronchopulmonary aspergillosis with low serum IgE levels in a child with cystic fibrosis. J Allergy Clin Immunol 1997; 100:431–432.
83. Schwartz HJ, Berger M, Hostoffer R. Allergic bronchopulmonary aspergillosis and common variable hypogammaglobulinemia in an adult male patient: case report. J Allergy Clin Immunol 1996; 98:708–710.
84. Skov M, Pressler T, Jensen HE, Hoiby N, Koch C. Specific IgG subclass antibody pattern to *Aspergillus fumigatus* in patients with cystic fibrosis with allergic bronchopulmonary aspergillosis (ABPA). Thorax 1999; 54:44–50.
85. Patterson R, Greenberger PA, Ricketti AJ, Roberts M. A radioimmunoassay index for allergic bronchopulmonary aspergillosis. Ann Intern Med 1983; 99:18–22.
86. Krasnick J, Greenberger PA, Roberts M, Patterson R. Allergic bronchopulmonary aspergillosis: serologic update for 1995. J Clin Lab Immunol 1995; 46:137–142.
87. Hearn VM, Proctor AG, Mackenzie DW. The preparation and partial characterization of antigenic fractions obtained from the mycelial walls of several *Aspergillus* species. J Gen Microbiol 1980; 119:41–49.

Marcel Dekker, Inc.
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88. Little SA, Warner JO. Improved diagnosis of allergic bronchopulmonary aspergillosis with gp66 (formerly antigen 7) of *Aspergillus fumigatus* for specific IgE detection. J Allergy Clin Immunol 1996; 98:55–63.
89. Crameri R, Hemmann S, Ismail C, Menz G, Blaser K. Disease-specific recombinant allergens for the diagnosis of allergic bronchopulmonary aspergillosis. Int Immunol 1998; 10:1211–1216.
90. Hemmann S, Nikolaizik WH, Schoni MH, Blaser K, Crameri R. Differential IgE recognition of recombinant *Aspergillus fumigatus* allergens by cystic fibrosis patients with allergic bronchopulmonary aspergillosis or *Aspergillus* allergy. Eur J Immunol 1998; 28:1155–1160.
91. Hemmann S, Ismail C, Blaser K, Menz G, Crameri R. Skin-test reactivity and isotype-specific immune responses to recombinant Asp f 3, a major allergen of *Aspergillus fumigatus*. Clin Exp Allergy 1998; 28:860–867.
92. Kawamura S, Maesaki S, Noda T, Hirakata Y, Tomono K, Tashiro T, Kohno S. Comparison between PCR and detection of antigen in sera for diagnosis of pulmonary aspergillosis. J Clin Microbiol 1999; 37:218–220.
93. Nikolaizik WH, Moser M, Crameri R, Little S, Warner JO, Blaser K, Schoni MH. Identification of allergic bronchopulmonary aspergillosis in cystic fibrosis patients by recombinant *Aspergillus fumigatus* I/a-specific serology. Am J Respir Crit Care Med 1995; 152:634–639.
94. Kiely JL, Spense L, Henry M, Hurley MF, Kelleher N, Bredin CP. Chest radiographic staging in allergic bronchopulmonary aspergillosis: relationship with immunological findings. Eur Respir J 1998; 12:453–456.
95. Lynch DA. Imaging of asthma and allergic bronchopulmonary mycosis. Radiol Clin North Am 1998; 36:129–142.
96. Shah A, Agarwal AK, Chugh IM. Hilar adenopathy in allergic bronchopulmonary aspergillosis. Ann Allergy Asthma Immunol 1999; 82:504–506.
97. Greenberger PA, Miller TP, Roberts M, Smith LL. Allergic bronchopulmonary aspergillosis in patients with and without evidence of bronchiectasis. Ann Allergy 1993; 70:333–338.
98. Fisher MR, Mendelson EB, Mintzer RA, Ricketti AJ, Greenberger PA. Use of linear tomography to confirm the diagnosis of allergic bronchopulmonary aspergillosis. Chest 1985; 87:499–502.
99. Angus RM, Davies ML, Cowan MD, McSharry C, Thomson NC. Computed tomographic scanning of the lung in patients with allergic bronchopulmonary aspergillosis and in asthmatic patients with a positive skin test to *Aspergillus fumigatus*. Thorax 1994; 49:586–589.
100. Panchal N, Pant C, Bhagat R, Shah A. Central bronchiectasis in allergic bronchopulmonary aspergillosis: comparative evaluation of computed tomography of the thorax with bronchography. Eur Respir J 1994; 7:1290–1293.
101. Logan PM, Muller NL. CT manifestations of pulmonary aspergillosis. Crit Rev Diagn Imaging 1996; 37:1–37.
102. Panchal N, Bhagat R, Pant C, Shah A. Allergic bronchopulmonary aspergillosis: the spectrum of computed tomography appearances. Respir Med 1997; 91:213–219.
103. Tamura A, Hebisawa A, Kurashima A, Kawabe Y, Machida K, Yotsumoto H, Mori

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

M. The use of bronchofiberscopy for diagnosis of allergic bronchopulmonary aspergillosis. Intern Med 1997; 36:865–869.
104. Aubry MC, Fraser R. The role of bronchial biopsy and washing in the diagnosis of allergic bronchopulmonary aspergillosis. Mod Pathol 1998; 11:607–611.
105. Patterson R, Greenberger PA, Lee TM, Liotta JL, O'Neill EA, Roberts M, Sommers H. Prolonged evaluation of patients with corticosteroid-dependent asthma stage of allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 1987; 80:663–668.
106. Wang JL, Patterson R, Rosenberg M, Roberts M, Cooper BJ. Serum IgE and IgG antibody activity against *Aspergillus fumigatus* as a diagnostic aid in allergic bronchopulmonary aspergillosis. Am Rev Respir Dis 1978; 117:917–927.
107. Ricketti AJ, Greenberger PA, Patterson R. Serum IgE as an important aid in management of allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 1984; 74:68–71.
108. van Rens MT, Vernooy-Jeras R, Merton-de Ridder M, van Velzen-Blad H, van den Bosch JM. Detection of immunoglobulins G and A to *Aspergillus fumigatus* by immunoblot analysis for monitoring *Aspergillus*-induced lung diseases. Eur Respir J 1998; 11:1274–1280.
109. Denning DW, Van Wye JE, Lewiston NJ, Stevens DA. Adjunctive therapy of allergic bronchopulmonary aspergillosis with itraconazole [see comments]. Chest 1991; 100:813–819.
110. Nepomuceno IB, Esrig S, Moss RB. Allergic bronchopulmonary aspergillosis in cystic fibrosis: role of atopy and response to itraconazole. Chest 1999; 115:364–370.
111. Fitzsimons EJ, Aris R, Patterson R. Recurrence of allergic bronchopulmonary aspergillosis in the posttransplant lungs of a cystic fibrosis patient. Chest 1997; 112: 281–282.
112. Tomee JF, Koeter GH, Hiemstra PS, Kauffman HF. Secretory leukoprotease inhibitor: a native antimicrobial protein presenting a new therapeutic option? Thorax 1998; 53:114–116.
113. Boulet LP, Chapman KR, Cote J, Kalra S, Bhagat R, Swystun VA, Laviolette M, Cleland LD, Deschesnes F, Su JQ, DeVault A, Fick RB, Jr., Cockcroft DW. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response [see comments]. Am J Respir Crit Care Med 1997; 155:1835–1840.
114. Demoly P, Bousquet J. Anti-IgE therapy for asthma [editorial; comment] [published erratum appears in Am J Respir Crit Care Med 1997; 156(5):1707]. Am J Respir Crit Care Med 1997; 155:1825–1827.
115. Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, Fick RB, Jr., Boushey HA. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects [see comments]. Am J Respir Crit Care Med 1997; 155:1828–1834.
116. Fahy JV, Boushey HA. Targeting IgE with monoclonal antibodies: the future is now [editorial; comment]. Clin Exp Allergy 1998; 28:664–667.

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19

IgE and Its Role in Parasitic Helminth Infection

Implications for Anti-IgE-Based Therapies

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I. Introduction

This report will detail current knowledge of the role of IgE in mediating immunity to helminth parasites. Because IgE responses to helminth infections differ based on chronicity of infection, the life cycle of the parasite, and the parasite's location within the host, it is difficult to apply data from one parasite to those of another, particularly in theorizing about the potential effects of anti-IgE therapy. The report will focus, in particular, on schistosomiasis and strongyloidiasis, for which most observational and experimental data have been accumulated to support a protective role for IgE. The latter infection is of particular relevance because of its unusual capacity to replicate within the human host, so-called auto-infection. Although most of the discussion will rely on data from human studies, much of this information is incomplete, and, where appropriate, observations from experimental animal models will be included.

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II. Natural History of the Immune Response to Human Helminth Infections

Helminth infections have complex life cycles that involve several developmental stages within the human host. The immune response to helminth parasites involves multiple effector pathways directed against different parasite stages that generally occur simultaneously within the same host. A useful paradigm with which to understand the immune response to helminth infections, and the changes that these responses undergo over time, is to divide helminth infections into acute and chronic.

A. Acute

Helminth infections may follow a short period of exposure or infrequent exposure. Acute infections may affect the following groups: (1) primary infections in experimental volunteers (1,2) or temporary travelers or expatriates in endemic areas with low-level and infrequent exposure (3); (2) young children living in endemic areas (4,5); (3) populations that have migrated to an endemic area from a nonendemic area (4,6); (4) nonmigrant populations that have become exposed en masse to transmission due to ecological changes (7,8); and (5) inhabitants of areas where parasite transmission is seasonal (9). The classic examples of acute helminth infections are reported in expatriates with relatively short exposure histories and who frequently develop clinically apparent allergic reactions (e.g., urticarial rashes) (10). Acute infections are associated with parasite-specific immunity, which is characterized by a mixed Th1/Th2 cytokine phenotype (11–13), marked eosinophilia, and elevated levels of parasite-specific IgE (3).

B. Chronic

To maintain parasite populations, most helminth parasites must maintain a state of persistent infectiousness within the human host. As host morbidity is closely related to parasite burden, most natural helminth infections of humans are likely to have co-evolved, with their hosts, mechanisms to maintain active infections but control parasite numbers. Primarily, there is the need to control the allergic reactions that are so typical of early/acute infections. Allergic phenomena are rare in individuals with long-standing chronic infections (14), and the immune response differs from the acute phenotype by a more polarized Th2 response (13,15,16) and the secretion of significant amounts of immunosuppressive cytokines such as IL-10 and TGF-β (16–18). Levels of total IgE are significantly higher in chronic infections with proportionately less parasite-specific IgE (3). High levels of polyclonal and parasite-specific IgG4 are also typical (19,20).

III. Role of IgE in Human Helminth Infections

Because of the marked differences in the immune responses observed during acute and chronic helminth infections, the potential protective role of IgE during the two stages of infection will be addressed separately.

A. Acute

The vast majority of individuals exposed to low infective doses of helminth parasites remain asymptomatic, although parasitological evidence of parasite patency may be evident (e.g., presence of eggs of *Schistosoma* spp. or geohelminth parasites). Generally, clinically apparent infections follow relatively high infective doses (21), although the presence of detectable IgE at the time of presentation is not invariable.

Experimental human inoculations with low doses of hookworm (e.g., 50 infective larvae) have been shown to result in marked eosinophilia but only small increases in total and parasite-specific IgE (2). In another human experiment, IgE levels increased only after subsequent infections, becoming detectable only after the third or fourth infection (1). Observations in acute schistosomiasis noted that the magnitude of IgG and IgE responses was related closely to the intensity of infection (21).

Many allergic syndromes have been reported in individuals in the acute stages of helminth infection. Typical examples are:

1. Wheezing or urticaria during the early migratory phase of infections with *Ascaris* spp., hookworm spp., *Strongyloides stercoralis*, and *Schistosoma* spp. (14).
2. Angioedematous Calabar swellings in *Loa loa* infections (22).
3. Tropical pulmonary eosinophilia in *Wuchereria bancrofti* infection (23).
4. Larva currens in *S. stercoralis* infection (24).

The underlying signs and symptoms associated with these syndromes suggest immediate hypersensitivity (e.g., wheeze, urticaria) and are often accompanied by marked eosinophilia.

Because many of these ''allergic'' reactions are directed against invasive larvae and are accompanied by marked local tissue eosinophilia, such reactions may serve to kill or immobilize larvae. In such reactions, crosslinking of mast cell–bound IgE by parasite antigens may be important in local eosinophil recruitment. Additionally, coating of parasites by antibodies including IgE may be important in antibody-dependent cellular cytotoxicity (ADCC) by eosinophils (25).

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B. Chronic

In many endemic areas, first infection with schistosomes occurs at a young age (approximately 4 years) (4). Because of early exposure and infection, older children and adults can be considered to have chronic infections; however, it should be remembered that although younger children may have hyperreactive immune responses to schistosome antigens (26), these undergo profound changes through later childhood and adulthood (27,28). Numerous observational field studies of human schistosomiasis have indicated a protective role for IgE.

Investigation of the factors associated with resistance to reinfection after praziquantel treatment of *Schistosoma mansoni*– and *S. haematobium*–infected populations has demonstrated correlations between parasite-specific IgE levels and resistance in a number of different studies (29–33). The mechanism by which IgE has been proposed to act is ADCC reactions in which IgE-coated parasites are attacked by effector cells including eosinophils, macrophages, and platelets (34).

The use of infection-reinfection studies to infer a protective role for parasite-specific IgE has a number of problems. First, a number of similar antigen preparations derived from different parasite stages were used to measure parasite-specific IgE in most studies, and there has been no consistency in the antigens associated with protection (29–33). Some studies have attributed protection to IgE directed against adult worm antigens (29,31,33), egg antigens (29), and/or schistosomula (30,33). In one study, anti-adult IgE was protective in one study population but not another, and negative associations between resistance to reinfection were observed for other IgE-specific parasite antigens (31). While IgE has been identified in some studies as the primary protective antibody, in other studies it has been identified as one of several immunoglobulin isotypes that correlate with resistance to reinfection including IgA (35) and IgG1 (4,36). There is evidence also that praziquantel treatment itself may alter the posttreatment immune response (37–39). Praziquantel-induced killing of adult schistosomes is an immune-dependent phenomenon (40), and detection of raised levels of IgE directed against adult worms in resistant individuals may merely reflect the enhanced Th2 responses (41) associated with this treatment.

A principal tenet of the IgE-protection hypothesis in schistosomiasis is that immunity is slowly acquired (26,28), is dependent on the period of exposure (e.g., age-dependent in endemic areas), and may only be partial (42). These factors have been proposed to explain the characteristic age-intensity curves in which peak infection levels occur in late childhood. The decline in intensity in early adulthood that cannot be explained sufficiently by changes in exposure is taken as evidence of age-acquired immunity (26,28). Recent immunological studies of communities with recently acquired exposure, however, demonstrate age-intensity profiles similar to those seen in endemic areas (8,28,43). Such observations argue for the importance of other age-dependent resistance mechanisms, including

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changing physiology (e.g., hormonal changes at puberty) or age-dependent barriers to infection (e.g., thicker skin) (27,43,44). The balance of evidence suggests that protective mechanisms are more complicated than previously thought, and although there probably is a role for Th2 immunity in host protection, such protective responses are likely to be multifactorial, heterogeneous between individuals, and not dependent on any single effector pathway such as IgE.

A consistent observation from the infection-reinfection studies of human schistosomiasis is that levels of parasite-specific IgE and IgG4, which are both high at the time of maximal infection intensity, become dissociated with increasing age. It has been suggested that IgG4 antibodies block IgE-mediated inflammation and that the decline in IgG4 levels with age leads to an unmasking of IgE-mediated immunity (26,29,45). Other antibody isotypes, apart from IgG4, including IgM and IgG2, have also been implicated as blocking antibodies (26,32,46). Levels of specific IgM and IgG2 have similar epidemiological correlations with IgE and are capable of blocking IgG1- and IgE-mediated killing of schistosomula in ADCC reactions in vitro (4,26). The presence of these blocking antibodies has been associated with susceptibility to reinfection with schistosomiasis following chemotherapy (26,47). Such blocking antibodies may prevent IgE-mediated larval killing by binding to reaginic epitopes on parasite antigens and prevent either binding of free IgE to the same epitopes or antigen crosslinking of mast cell–bound IgE.

Chronic infections with tissue-invasive helminth infections are associated with very high levels of serum IgE that may reach greater than one hundred times the normal range (14). The proportion of IgE that is parasite specific diminishes in chronic infections and constitutes less than 10% of total IgE (48). In addition, the IgE of helminth-infected subjects recognizes a very wide range of parasite antigens (49). Excess production of large quantities of nonspecific IgE may have consequences with regard to parasite-directed inflammatory responses. Nonspecific IgE may saturate FcεRI receptors on basophils and mast cells and prevent antigen triggering of mast cell degranulation (14). Likewise, saturation of FcεR11 with nonspecific IgE on antigen-presenting cells may prevent optimal IgE-dependent antigen focusing and presentation to T cells (50).

Human infection with *S. stercoralis* is a classic example of a chronic helminthiasis. Unlike other helminth infections that require repeated exposure to maintain parasite numbers in the human host, infection with *S. stercoralis* can be maintained for many years after an initial exposure through internal autoinfection. Rhabditiform larvae that develop from eggs in the submucosa of the small intestine have the capacity to develop rapidly into filariform infective larvae that can reinvade the host through the mucosa of the large intestine and maintain the infection indefinitely. Most individuals with strongyloidiasis remain asymptomatic and are able to control their parasite burdens effectively—it may be impossible to detect parasite larvae by stool examination in *Strongyloides*-seropositive individuals. However, a few individuals may develop hyperinfection that can lead to

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larval invasion of all body tissues and results occasionally in death. *Strongyloides* hyperinfection is often accompanied by some degree of immunosuppression that has been associated with hypogammaglobulinemia (51,52), hematological malignancies, long-term steroid therapy, or infections with the human T-lymphotropic virus I (HTLV-I).

Potential host-protective mechanisms identified from experimental animal models of *Strongyloides* infection include IL-5–dependent (53–55), IgM antibody–dependent (56), and mast cell–mediated (57) mechanisms. Studies of subjects with various clinical and parasitological forms of chronic infection do not support a role for specific IgE in host protection (58–60), although a recent study (60) demonstrated differential patterns of IgA- and IgE-specific antigen recognition between individuals with parasite-positive and parasite-negative stools.

The most interesting data supporting a role for IgE in host protection against *S. stercoralis* infection derive from populations in which concurrent infections with HTLV-I are relatively frequent, particularly in the Caribbean and the southern islands of Japan. These studies have demonstrated an epidemiological relationship between *S. stercoralis* infection and concurrent HTLV-I infection (61–63). Carriers of HTLV-I infection do not have an increased risk of acquiring *S. stercoralis* infection (e.g., seropositive for *S. stercoralis*) but do have an increased risk of a parasitologically detectable infection, implying that such co-infected individuals are less capable of controlling parasite numbers (61). A number of studies have demonstrated decreased levels of total IgE in co-infected individuals (61,63–65). A recent study investigating co-infection of HTLV-I with *S. stercoralis* was able to demonstrate high spontaneous rates of IFN-γ secretion in such co-infected subjects compared with those with either infection alone or neither infection (65). The same study demonstrated reduced levels of specific IgE and a negative correlation between spontaneous IFN-γ secretion and total IgE levels (65). The authors proposed that the secretion of high background levels of IFN-γ by HTLV-I carriers was suppressing IL-4 secretion and IgE synthesis, leading to a specific immune defect that might lead to hyperinfection.

There are, however, a number of problems with the epidemiological and immunological data from these co-infection studies that cast doubt on the putatively protective role of IgE in strongyloidiasis. Many of the studies include older individuals with HTLV-I infection who are likely to have more advanced disease (65,66). Furthermore, HTLV-I infection is associated with multiple immune abnormalities affecting both humoral and cellular immune responses (67) including spontaneous secretion of multiple cytokines including IFN-γ (68).

Adult T-cell leukemia (ATL), the most common sequela of HTLV-I infection, occurs in up to 4% of infected individuals and is preceded by a long latent period of many years (69). ATL may take many forms and is preceded by a preleukemic phase that may or may not progress to more advanced disease (70). HTLV-I infection in older individuals is, therefore, more likely to be accompa-

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nied by an unbalanced immune response that may lead to increased susceptibility to different infections (62) including patent *S. stercoralis* infection (66) and, occasionally, *Strongyloides* hyperinfection. The relative deficiency in IgE levels observed in some studies in HTLV-I carriers co-infected with *S. stercoralis* is most likely an artifact of global IL-4 suppression and probably a more profound state of immunodeficiency. For example, in one study co-infections were associated not only with significant suppression of specific IgE but also with levels of specific IgG1 and IgA (71).

Coinfected individuals are less likely to respond to anthelmintic therapy with parasitological cure (72,73). Anthelmintic treatments such as albendazole, which are widely used in many regions, cure up to 95% of *S. stercoralis* infections after a single treatment course (74,75) and 100% after two or more courses (76). Studies of the interaction between HTLV-I and *S. stercoralis* conducted in co-endemic areas are more likely to identify, therefore, *S. stercoralis* infection among those individuals less responsive to anthelmintic treatment (e.g., HTLV-I–associated immunosuppression), leading to a biased assessment of the role of IgE (and other immune markers) in protective immunity against *S. stercoralis*.

There is little evidence for a role of IgE in host protection against chronic infections with other geohelminth parasites such as *Trichuris trichiura* (77) and *Ascaris lumbricoides* (78,79). Of interest, the *Trichuris* dysentery syndrome, observed occasionally in children with heavy infections, demonstrated marked mucosal mastocytosis and infiltration by IgE-staining cells but with little impact on intestinal parasite burden (80). There is some epidemiological evidence that IgE may be protective in human hookworm infections (81,82), but, as with schistosomiasis, there are significant problems in the interpretation of these correlational field data.

IV. Does IgE Have a Protective Role Against Helminth Infections?

The balance of evidence indicates that parasite-specific IgE may contribute to immediate hypersensitivity phenomena and parasite-directed inflammatory reactions (ADCC) in human helminth infections. IgE-induced mast cell degranulation may play a significant role in the allergic phenomena associated with early or acute infections in recruitment of effector cells to the tissue sites of helminth parasites, although the effectiveness of these mechanisms in parasite killing is not clear. The intense inflammation that results is the principal cause of the tissue pathology and clinical disease associated with these infections. In the absence of IgE, it is probable that other immunoglobulin isotypes can participate in ADCC reactions with similar efficiencies. Observations from primary infections with helminths in humans indicate that IgE is unlikely to have a critical role in host

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protection against helminth parasites. In the case of chronic helminth infections such as schistosomiasis, IgE may function in conjunction with other Th2 effector mechanisms to control parasite burdens and prevent superinfection. The massive increases in nonspecific IgE secretion in chronic infections may actually inhibit potentially protective or host-damaging IgE-mediated immune reactions. The identification of IgE as a marker of protective immunity in schistosomiasis and strongyloidiasis is most likely a reflection of an underlying Th2 bias capable of inducing multiple protective immune effector arms.

A protective role for IgE against helminth parasites is supported by some animal studies of rats infected with filarial parasites (83), *T. spiralis* (84), or *S. mansoni* (85). In contrast, however, are the findings that mice incapable of making IgE (using anti-IL-4 monoclonal antibodies, IL-4/IL-13 knockouts, or IL-4R knockouts) can respond to vaccination against *S. mansoni*, suggesting that IgE alone is not mediating protection to schistosome infection (86). Similarly, in mice with a targeted deletion of the IgE gene, although primary infection with *S. mansoni* was associated with higher worm burdens, resistance to challenge infection was no different than in mice with the normal IgE gene (87). Consistent with these findings are the results in IgE-suppressed mice in which resistance to *S. japonicum* was no different than that in control mice with normal IgE levels (88).

Intestinal helminths have been used as the prototypical parasite infections to demonstrate the importance of IgE in mediating protection. Indeed, it has been demonstrated that passive transfer of IgE antibodies can mediate the rapid expulsion phenomenon in rats infected with *T. spiralis* (89) and that diminished levels of IgE are associated with dissemination of *Strongyloides stercoralis* (61,90) in the context of HTLV-I coinfection. With the elucidation of the cytokine control of the IgE response (e.g., IL-4 and IL-13), however, and the fact the these cytokine responses exist in the context of a broader (Th2 or Type 2) response, the IgE response itself may be merely an indicator of a more general response, whose many different components effect the protective response (reviewed in Refs. 91–94).

V. Animal Models of Impact of Anti-IgE on Helminth Infections

The impact of anti-IgE antibodies on parasite burdens has been examined in animal models of infections with *S. mansoni*, the lung fluke *Paragonimus wetermani*, and *Strongyloides ratti*. Despite undetectable levels of IgE posttreatment, treatment with anti-IgE resulted in reduced parasite burdens in mice infected with *P. westermani* (95) and decreased worm burden and a decrease in the number of eggs produced per worm in mice infected with *S. mansoni* (96). Experimental infections with *S. ratti* were not affected by anti-IgE treatment (97). Potential

explanations for the protective effect of anti-IgE in these models include (1) suppression of nonspecific IgE levels, leading to more parasite-specific and focused CD23-dependent T-cell signaling (95), and (2) IgE-dependent cellular cytotoxicity to parasitic worms mediated by enhanced binding of parasite-specific IgE to FcεRII/CD23 on macrophages and eosinophils.

VI. Potential Risks in Travelers to Endemic Areas

Most travelers to helminth-endemic regions have brief and short exposures to helminth transmission. Generally, such infections remain asymptomatic and undetected unless routine screening is performed and infection is suggested by either unexplained eosinophilia or detection of parasite larvae or eggs in the stool. Current evidence indicates that primary infections of this sort are self-limiting and, when not treated, will disappear as the adult worms die. More unusual situations arise when the primary exposure is prolonged or intense, resulting in larger infectious innocula. Infections in such individuals are more likely to be detected because of the development of typical symptoms or signs. Severe pathology, typically associated with large parasite burdens acquired over many years of continuous exposure, is seen very rarely.

The risks associated with anti-IgE–directed treatment for allergic disease in individuals with light infections are likely to be minimal. Assuming a protective role for IgE, possible effects of anti-IgE therapy in individuals exposed to infection under these circumstances might include (1) larger adult worm populations (more surviving larvae)—not supported by experimental models (95), (2) prevention of allergic reactions to invasive larvae—a likely positive outcome given the effects of anti-IgE on allergic inflammation, and (3) a perhaps increased rate of asymptomatic infection—this might result from the survival of very small infectious innocula to adulthood and more silent infections due to the antiallergic effects of IgE-based therapies. There is little evidence to justify concerns over an increased risk of *Strongyloides* hyperinfection (see Sec. III. B); however, added caution is warranted in individuals taking concurrent oral steroid therapy for allergic disease or who have other immunodeficiencies that, independently, are risk factors for *Strongyloides* hyperinfection.

VII. Potential Risks in Individuals with Undiagnosed Helminth Infections

Anti-IgE may be administered unwittingly to individuals with undiagnosed helminth infections. Under these circumstances, helminth parasite burdens are likely to be light, and therefore administration of anti-IgE should have no adverse effects. An attempt to diagnose and treat cryptic helminth infections should, how-

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ever, be attempted in all individuals with a history of significant exposure in any nonindustrialized country. Individuals with histories of potential exposure should be screened for blood eosinophilia and stool parasites. The risks of *Strongyloides* hyperinfection have been discussed in Sec. VI. Administration of anti-IgE in helminth-endemic regions to subjects with chronic helminth infections and continued exposure to transmission carries a theoretical risk of superinfection that may lead to significant clinical disease due to large increases in parasite burdens. Findings from experimental animal models do not support such an outcome (95–97). In any case, such individuals and many others with lighter infections are likely to have total IgE levels in excess of 1000 IU/mL and are not, therefore, candidates for anti-IgE therapy at currently recommended dosage regimens.

VIII. Conclusions

Although IgE is regarded as an important immune effector mechanism against human helminth parasites, evidence to support such a role is not strong. IgE may have a role in controlling parasite numbers in chronic helminth infections such as schistosomiasis, but redundancy in the immune response would suggest that such a role is likely to be duplicated by other immunoglobulin molecules. The typical features of helminth infections, such as raised IgE, eosinophilia, and mastocytosis, reflect a heightened state of Th2 activation and polarization. In this chapter, the potential protective role of IgE in schistosomiasis and strongyloidiasis has been discussed. The overall balance of evidence does not support a primary role for this immunoglobulin in host protection in either of these infections. The potential risks with respect to enhanced helminth parasite survival in travelers to endemic areas that might be receiving anti-IgE therapy are likely to be very low. Likewise, there are almost no probable risks of treatment in individuals harboring nonreplicative cryptic helminth infections. In the case of *Strongyloides stercoralis* infections, the risks of anti-IgE therapy are also likely to be low, but added caution is warranted in individuals with underlying immunodeficiencies or who are taking oral steroids.

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References

1. Ogilvie BM, Bartlett A, Godfrey R, Turton T, Worms M, Yeates R. Antibody responses in self infections with *Necator americanus*. Trans R Soc Trop Med Hyg 1978; 72:66–71.

2. Maxwell C, Hussain R, Nutman TB, Poindexter RW, Little MD, Schad GA, Ottesen EA. The clinical and immunologic responses of normal human volunteers to low dose hookworm (*Necator americanus*) infection. Am J Trop Med Hyg 1987; 37: 126–134.
3. McCarthy JS, Ottesen EA, Nutman TB. Onchocerciasis in endemic and nonendemic populations: differences in clinical presentation and immunologic findings. J Infect Dis 1994; 170:736–741.
4. Butterworth AE, Corbett EL, Dunne DW, Fulford AJC, Kimani G, Gachuhi RK, Klumpp R, Mbugua G, Ouma JH, Siongok TK, Sturrock RF. Immunity and morbidity in human schistosomiasis. In: McAdam KPWJ, ed. Frontiers of Infectious Diseases: New Strategies in Parasitology. Edinburgh: Churchill Livingstone, 1989:193–210.
5. Luty AJF, Downham MD, Whitworth JAG, Morgan D, McNicholas A, Taylor DW. Immunological studies on onchocerciasis in Sierra Leone. 1. Pretreatment baseline data. Trop Med Parasitol 1990; 41:371–375.
6. Partono F, Purnomo, Pribaldi W, Soewarta A. Epidemiological and clinical features of *Brugia malayi* in a newly established village, Karkuak, West Flores, Indonesia. Am J Trop Med Hyg 1978; 27:910–915.
7. Talla I, Kongs A, Verle P, Belot J, Sarr S, Coll AM. Outbreak of intestinal schistosomiasis in the Senegal River basin. Ann Soc Belge Med Trop 1990; 70:173–180.
8. Stelma FF, Talla I, Polman K, Niang M, Sturrock RF, Deelder AM, Gryseels B. Epidemiology of *Schistosoma mansoni* infection in a recently exposed community in northern Senegal. Am J Trop Med Hyg 1993; 49:701–706.
9. Gelpi AP, Mustafa A. Seasonal pneumonitis with eosinophilia: a study of larval ascariasis in Saudi Arabs. Am J Trop Med Hyg 1967; 16:646–657.
10. Jarrett EEE, Miller HRP. Production and activities of IgE in helminth infection. Prog Allergy 1982; 31:178–233.
11. Mahanty S, Abrams JS, King CL, Limaye AP, Nutman TB. Parallel regulation of IL-4 and IL-5 in human helminth infections. J Immunol 1992; 148:3567–3571.
12. Dimock KA, Eberhard ML, Lammie PJ. Th1-like antifilarial immune responses predominate in antigen-negative persons. Infect Immun 1996; 64:2962–2967.
13. Soboslay PT, Geiger SM, Weiss N, Banla M, Luder CG, Dreweck CM, Batchassi E, Boatin BA, Stadler A, Schulz-Key H. The diverse expression of immunity in humans at distinct states of *Onchocerca volvulus* infection. Immunology 1997; 90: 592–599.
14. Ottesen E. Parasite infections and allergic reaction—how each affects the other. In: Weiss EB, Segal MS, Stein M, eds. Bronchial Asthma: Mechanisms and Therapeutics, 2d ed. New York: Little, Brown and Co., 1985:522–527.
15. King CL, Mahanty S, Kumaraswami V, Abrams JS, Regunathan J, Jayaraman K, Ottesen EA, Nutman TB. Cytokine control of parasite-specific anergy in human lymphatic filariasis. Preferential induction of a regulatory T helper type 2 lymphocyte subset. J Clin Invest 1993; 92:1667–1673.
16. King CL, Medhat A, Malhotra I, Nafeh M, Helmy A, Khaudary J, Ibrahim S, El-Sherbiny M, Zaky S, Stupi RJ, Brustoski K, Shehata M, Shata MT. Cytokine control of parasite-specific anergy in human urinary schistosomiasis: IL-10 modulates lymphocyte reactivity. J Immunol 1996; 156:4715–4721.

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270 Madison Avenue, New York, New York 10016

17. Soboslay PT, Luder CGK, Riesch S, Geiger SM, Banla M, Batchassi E, Stadler A. Regulatory effects of Th1-type (IFN-γ, IL-12) and Th2-type (IL-10, IL-13) cytokines on parasite-specific cellular responsiveness in *Onchocerca volvulus*-infected humans and exposed endemic controls. Immunology 1999; 97:219–225.
18. Doetze A, Satoguina J, Burchard G, Rau T, Fleischer B, Hoerauf A. Antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by Th3/Tr1-type cytokines IL-10 and TGF-β but not by a Th1 to Th2 shift. Int Immunol 2000; 12:623–630.
19. Hussain R, Poindexter RW, Ottesen EA. Control of allergic reactivity in human filariasis: predominant localization of blocking antibodies to the IgG4 subclass. J Immunol 1991; 148:2731–2737.
20. Mahanty S, Day KP, Alpers MP, Kazura JW. Antifilarial IgG4 antibodies in children from filaria-endemic areas correlate with duration of infection and are dissociated from antifilarial IgE antibodies. J Infect Dis 1994; 170:1339–1343.
21. Hiatt RA, Sotomayor ZR, Sanchez G, Zambrana M, Knight WB. Factors in the pathogenesis of acute *Schistosomiasis mansoni*. J Infect Dis 1979; 139:659–666.
22. Nutman TB, Miller KD, Mulligan M, Ottesen EA. Loa loa infection in temporary residents of endemic regions: recognition of a hyperresponsive syndrome with characteristic clinical manifestations. J Infect Dis 1986; 154:10–18.
23. Neva FA, Ottesen EA. Tropical (filarial) eosinophilia. N Engl J Med 1978; 298: 1129–1131.
24. Neva FA. Biology and immunology of human strongyloidiasis. J Infect Dis 1986; 153:397–406.
25. Butterworth AE. Cell-mediated damage to helminths. Adv Parasitol 1984; 23:143–235.
26. Butterworth AE, Curry AJ, Dunne DW, Fulford AJ, Kimani G, Kariuki HC, Klumpp R, Koech D, Mbugua G, Ouma, JH, Roberts M, Thiongo FW, Capron A, Sturrock RF. Immunity and morbidity in human *Schistosomiasis mansoni.* Trop Georgr Med 1994; 46:197–208.
27. Fulford AJC, Webster M, Ouma JH, Kimani G, Dunne DW. Puberty and age-related changes in susceptibility to schistosome infection. Parasitol Today 1998; 14:23–26.
28. Hagan P, Ndhlovu PD, Dunne DW. Schistosome immunology: more questions than answers. Parasitol Today 1998; 14:407–412.
29. Hagan P, Blumenthal UJ, Dunn D, Simpson AJ, Wilkins HA. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. Nature 1991; 349:243–245.
30. Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. Eur J Immunol 1991; 21:2679–2686.
31. Dunne DW, Butterworth AE, Fulford AJC, Kariuki HC, Langley JG, Ouma JH, Capron A, Pierce RJ, Sturrock RF. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. Eur J Immunol 1992; 22:1483–1494.
32. Demeure EC, Rihet P, Abel L, Ouattara M, Bourgois A, Dessein AJ. Resistance to *Schistosoma mansoni* in humans: influence of IgE/IgG4 balance and IgG2 in immunity to reinfection after chemotherapy. J Infect Dis 1993; 168:1000–1009.

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33. Caldas IR, Correa-Oliveira R, Colosimo E, Carvalho OS, Massara CL, Colley DG, Gazzinelli G. Susceptibility and resistance to *Schistosoma mansoni* reinfection: parallel cellular and isotypic immunologica assessment. Am J Trop Med Hyg 2000; 62:57–64.
34. Capron M, Capron A. Immunoglobulin E and effector cells in schistosomiasis. Science 1994; 254:1876–1877.
35. Gryzch JM, Grezel D, Xu CB, Neyrinck JL, Capron M, Ouma JH, Butterworth AE, Capron A. IgA antibodies to a protective antigen in human *Schistosomiasis mansoni.* J Immunol 1993; 150:527–535.
36. Dessein AJ, Begley M, Demeure C, Caillol D, Fueri J, dos Reis MG, Andrade ZA, Prata A, Bina JC. Human resistance to *Schistosoma mansoni* is associated with IgG reactivity to a 37-Kda larval surface antigen. J Immunol 1988; 140:2727–2736.
37. Grogan JL, Kremsner PG, van Dam GJ, Deelder AM, Yazdanbakhsh M. Antischistosome IgG4 and IgE responses are affected differentially by chemotherapy in children versus adults. J Infect Dis 1996; 173:1242–1247.
38. Webster M, Fallon PG, Fulford AJ, Butterworth AE, Ouma JH, Kimani G, Dunne DW. Effect of praziquantel and oxamniquine treatment on human isotype responses to *Schistosoma mansoni*: elevated IgE to adult worm. Parasite Immunol 1997; 19: 333–335.
39. Mutapi F, Ndhlovu PD, Hagan P, Spicer JT, Mduluza T, Turner CM, Chandiwana SK, Woolhouse ME. Chemotherapy accelerates the development of acquired immune responses to *Schistosoma heamatobium* infection. J Infect Dis 1998; 178:289–293.
40. Fallon PG, Cooper RO, Probert AJ, Doenhoff MJ. Immune-dependent chemotherapy of schistosomiasis. Parasitology 1992; 105:S41.
41. Roberts M, Butterworth AE, Kimani G, Kamau T, Fulford AJC, Dunne DW, Ouma JH, Sturrock RF. Immunity after treatment of human schistosomiasis: association between cellular responses and resistance to reinfection. Infect Immun 1993; 61: 4984–4993.
42. Smithers SR, Terry RJ. Concomitant immunity. Adv Parasitol 1976; 14:399–428.
43. Naus CWA, Kimani G, Ouma JH, Fulford AJC, Webster M, van Dam GJ, Deelder AM, Butterworth AE, Dunne DW. Development of antibody isotype responses to *Schistosoma mansoni* in an immunologically naïve immigrant population: influence of infection duration, infection intensity, and host age. Infect Immun 1999; 67:3444–3451.
44. van Dam GJ, Stelma FF, Gryseels B, Falcao Ferreira ST, Talla I, Niang M, Rotmans JP, Deelder AM. Antibody response patterns against *Schistosoma mansoni* in a recently exposed community in Senegal. J Infect Dis 1996; 173:1232–1241.
45. Rihet P, Demeure CE, Dessein AJ, Bourgois A. Strong serum inhibition of specific IgE correlated to competing IgG4, revealed by a new methodology in subjects from a *S. mansoni* endemic area. Eur J Immunol 1992; 22:2063–2070.
46. Khalife J, Dunne DW, Richardson BA, Mazza G, Thorne KJ, Capron A, Butterworth AE. Functional role of human IgE subclasses in eosinophil-mediated killing of schistosomula of *Schistosoma mansoni*. J Immunol 1989; 142:4422–4427.
47. Butterworth AE, Bensted SR, Capron A, Capron M, Dalton PR, Dunne DW, Grzych JM, Kariuki HC, Khalife J, Koech D, Siongok TKA, Sturrock RF. Immunity in

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human *Schistosomiasis mansoni*: prevention by blocking antibodies of the expression of immunity in young children. Parasitology 1987; 94:281–300.
48. Dessaint J-P, Capron M, Bout D, Capron A. Quantitative determination of specific IgE antibodies to schistosome antigens and serum IgE levels in patients with schistosomiasis (*S. mansoni* or *S. haematobium*). Exp Immunol 1975; 20:427.
49. Hussain R, Ottesen EA. IgE responses in human filariasis. III. Specificities of IgE and IgG antibodies compared by immunoblot analysis. J Immunol 1985; 135:1415–1420.
50. Fujiwara H, Kikutani H, Suematsu S, Naka T, Yoshida K, Yoshida K, Tanaka T, Suemura M, Matsumoto N, Kojima S, Kishimoto T, Yoshida N. The absence of IgE antibody-mediated augmentation of immune responses in CD23-deficient mice. Proc Natl Acad Sci USA 1994; 91:6835–6839.
51. Brandt de Oliveira R. Severe strongyloidiasis associated with hypogammaglobulinemia. Parasite Immunol 1981; 3:165–169.
52. Shelhamer JH, Neva FA, Finn DR. Persistent strongyloidiasis in an immunodeficient patient. Am J Trop Med Hyg 1982; 31:746–751.
53. Korenaga M, Hitoshi Y, Yamaguchi N, Sato Y, Takatsu K, Tada I. The role of interleukin-5 in protective immunity to *Strongyloides venezuelensis* infection in mice. Immunology 1991; 72:502–507.
54. Korenaga M, Hitoshi Y, Takatsu K, Tada I. Regulatory effect of anti-interleukin-5 monoclonal antibody on intestinal worm burden in a primary infection with *Strongyloides venezuelensis* in mice. Int J Parasitol 1994; 24:951–957.
55. Nawa Y, Ishikawa N, Tsuchiya K, Horii Y, Abe T, Khan AI, Bing-Shi, Itoh H, Ide H, Uchiyama F. Selective effector mechanisms for the expulsion of intestinal helminths. Parasite Immunol 1994; 16:333–338.
56. Brigandi RA, Rotman HL, Yutanawiboonchai W, Leon O, Nolan TJ, Schad GA, Abraham D. *Strongyloides stercoralis*: role of antibody and complement in immunity to the third stage larvae in BALB/cByJ mice. Exp Parasitol 1996; 82:279–289.
57. Finkelman FD, Shea-Donohue T, Goldhill J, Sullivan CA, Morris SC, Madden KB, Gause WC, Urban JF. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. Annu Rev Immunol 1997; 15:505–533.
58. Sato Y, Takara M, Otsuru M. Detection of antibodies in strongyloidiasis by enzyme-linked immunosorbent assay (ELISA). Trans R Soc Trop Med Hyg 1985; 79:51–55.
59. Badaro R, Carvalho EM, Santos RB, Gam AA, Genta RM. Parasite-specific humoral responses in different clinical forms of strongyloidiasis. Trans R Soc Trop Med Hyg 1987; 81:149–150.
60. Atkins NS, Conway DJ, Lindo JF, Bailey JW, Bundy DAP. L3 antigen-specific antibody isotype responses in human strongyloidiasis: correlations with larval output. Parasite Immunol 1999; 21:517–526.
61. Robinson RD, Lindo JF, Neva FA, Gam AA, Vogel P, Terry SI, Cooper ES. Immunoepidemiologic studies of *Strongyloides stercoralis* and human T lymphotropic virus type I infections in Jamaica. J Infect Dis 1994; 169:692–696.
62. Marsh BJ. Infectious complications of human T cell leukemia/lymphoma virus type I infection. Clin Infect Dis 1996; 23:138–145.

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63. Hayashi J, Kishihara Y, Yoshimura E, Furusyo N, Yamaji K, Kawakami Y, Murakami H, Kashiwagi S. Correlation between human T cell lymphotropic virus Type-1 and *Strongyloides stercoralis* infections and serum immunoglobulin E responses in residents of Okinawa, Japan. Am J Trop Med Hyg 1997; 56:71–75.
64. Robinson RD, Lindo JF, Neva F. Interaction between HTLV-1 and *Strongyloides stercoralis* infection resulting in lowered IgE titres in Jamaica. West Indian Med J 1990; 39:35–36.
65. Neva FA, Filho JO, Gam AA, Thompson R, Freitas V, Melo A, Carvalho EM. Interferon-γ and interleukin-4 responses in relation to serum IgE levels in persons infected with human T lymphotropic virus Type I and *Strongyloides stercoralis*. J Infect Dis 1998; 178:1856–1859.
66. Nakada K, Yamaguchi K, Furugen S, Nakasone T, Nakasone K, Oshiro Y, Kohakura M, Hinuma Y, Seiki M, Yoshida M, Matutes E, Catovsky D, Ishii T, Takatsuki K. Monoclonal integration of HTLV-I proviral DNA in patients with strongyloidiasis. Int J Cancer 1987; 40:145–148.
67. Hollsberg P, Hafler DA. Pathogenesis of disease induced by human lymphotropic virus type I infection. N Engl J Med 1993; 328:1173–1182.
68. Salahuddin SZ, Markham PD, Lindner SG, Gootenberg J, Popovic M, Hemmi H, Sarin PS, Gallo RC. Lymphokine production by cultured human T cells transformed by human T-cell leukemia-lymphoma virus-I. Science 1984; 223:703–707.
69. Murphy EL, Hanchard B, Figueroa JP, Gibbs WN, Lofters WS, Campbell M, Goedert JJ, Blattner WA. Modelling the risk of adult T-cell leukemia/lymphoma in persons infected with human T-lymphotropic virus type I. Int J Cancer 1989; 43:250–253.
70. Kinoshita K, Amagasaki T, Ikeda S, Suzuyama J, Toriya K, Nishino K, Tagawa M, Ichimaru M, Kamihira S, Yamada Y, Momita S, Kusano M, Morikawa T, Fujita S, Ueda Y, Ito N, Yoshida M. Preleukemic state of adult T cell leukemia: abnormal T lymphocytosis induced by human adult T cell leukemia-lymphoma virus. Blood 1985; 66:120–127.
71. Atkins NS, Lindo JF, Lee MG, Hanchard B, Robinson RD, Bundy DAP. Immunomodulatory effects of concurrent HTLV-I infection in strongyloidiasis. J AIDS 1998; 18:188–190.
72. Sato Y, Shiroma Y, Kiyuna S, Toma H, Kobayashi J. Reduced efficacy of chemotherapy might accumulate concurrent HTLV-I infection among strongyloidiasis patients in Okinawa, Japan. Trans R Soc Trop Med Hyg 1994; 88:59.
73. Toma H, Sato Y, Shiroma Y, Kobayashi J, Shimabukuro I, Takara M. Comparative studies on the efficacy of three anthelminthics on treatment of human strongyloidiasis in Okinawa, Japan. SE Asian J Trop Med Public Health 2000; 31:147–151.
74. Archibald LK, Beeching NJ, Gill GV, Bailey JW, Bell DR. Albendazole is effective treatment for chronic strongyloidiasis. Q J Med 1993; 86:191–195.
75. Pitisuttithum P, Supanaranond W, Chindanond D. A randomized comparative study of albendazole and thiabendazole in chronic strongyloidiasis. SE Asian J Trop Med Public Health 1995; 26:735–738.
76. Pungpak S, Bunnag D, Chindanond D, Radmoyos B. Albendazole in the treatment of strongyloidiasis. SE Asian J Trop Med Public Health 1987; 18:207–210.
77. Needham CS, Bundy DAP, Lillywhite JE, Didier JM, Simmons I, Bianco AE. The

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relationship between *Trichuris trichiura* transmission intensity and the age-profiles of parasite-specific antibody isotypes in two endemic communities. Parasitology 1992; 105:272–283.
78. Palmer DR, Hall A, Haque R, Anwar KS. Antibody isotype responses to antigens of *Ascaris lumbricoides* in a case-control study of persistently heavily infected Bangladeshi children. Parasitology 1995; 111:385–393.
79. Cooper PJ, Chico M, Sandoval C, Espinel I, Guevara A, Kennedy MW, Urban JR Jr, Griffin GE, Nutman TB. Human infection with *Ascaris lumbricoides* is associated with a polarized cytokine phenotype. J Infect Dis 2000; 182:1207–1213.
80. Cooper ES, Spencer J, Whyte-Alleng CAM, Cromwell O, Whitney P, Venugopal S, Bundy DA, Haynes B, MacDonald TT. Immediate hypersensitivity in colon of children with chronic *Trichuris trichiura* dysentery. Lancet 1991; 338:1104–1107.
81. Pritchard DI, Quinnell RJ, Walsh EA. Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. Parasite Immunol 1995; 17:71–75.
82. Quinnell RJ, Woolhouse MEJ, Walsh EA, Pritchard DI. Immunoepidemiology of human necatoriasis: correlations between antibody responses and parasite burdens. Parasite Immunol 1995; 17:313–318.
83. Gusmao RD, Stanley AM, Ottesen EA. *Brugia pahangi*: immunologic evaluation of the differential susceptibility of filarial infection in inbred Lewis rats. Exp Parasitol 1981; 52:147–159.
84. Grove DI, Warren KS. Effects on murine trichinosis of niridazole, a suppressant of cellular but not humoral immunological responses. Ann Trop Med Parasitol 1976; 70:449–453.
85. Capron A, Dessaint JP. Effector and regulatory mechanisms in immunity to schistosomes: a heuristic view. Annu Rev Immunol 1985; 3:455–476.
86. Sher A, Coffman RL, Hieny S, Cheever AW. Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibody fails to affect immunity against *Schistosoma mansoni* in the mouse. J Immunol 1990; 145:3911–3916.
87. King CL, Xianli J, Malhotra I, Liu S, Mahmoud AA, Oettgen HC. Mice with a targeted deletion of the IgE gene have increased worm burdens and reduced granulomatous inflammation following primary infection with *Schistosoma mansoni*. J Immunol 1997; 158:294–300.
88. Watanabe N, Janecharut T, Kojima S, Ovary Z. Acquired resistance to *Schistosoma japonicum* in IgE-deficient SJA/9 mice immunized with irradiated cercariae. Int Arch Allergy Immunol 1993; 102:191–194.
89. Ahmad A, Wang CH, Bell RG. A role for IgE in intestinal immunity. Expression of rapid expulsion of *Trichinella spiralis* in rats transfused with IgE and thoracic duct lymphocytes. J Immunol 1991; 146:3563–3570.
90. Newton RC, Limpuangthip P, Greenberg S, Gam A, Neva FA. *Strongyloides stercoralis* hyperinfection in a carrier of HTLV-I virus with evidence of selective immunosuppression. Am J Med 1992; 92:202–208.
91. Urban JF Jr, Madden KB, Svetic A, Cheever A, Trotta PP, Gause WC, Katona IM, Finkelman FD. The importance of Th2 cytokines in protective immunity to nematodes. Immunol Rev 1992; 127:205–220.
92. Else KJ, Finkelman FD, Maliszewski CR, Grencis RK. Cytokine-mediated regulation of chronic intestinal helminth infection. J Exp Med 1994; 179:347–351.

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93. Grencis RK. Enteric helminth infection: immunopathology and resistance during intestinal nematode infection. Chem Immunol 1997; 66:41–61.
94. Grencis RK. Th2-mediated host protective immunity to intestinal nematode infections. Phil Trans R Soc Lond B Biol Sci 1997; 352:1377–1384.
95. Shin M-H, Min H-K. Effects of anti-IgE on serum IgE, FcεRII/CD23 expression on splenic B cells and worm burden in mice infected with *Paragonimus westermani*. Kor J Parasitol 1997; 35:47–54.
96. Amiri P, Haak-Frendscho M, Robbins K, McKerrow JH, Stewart T, Jardieu P. Anti-immunoglobulin E treatment decreases worm burden and egg production in *Schistosoma mansoni*-infected normal and interferon-γ knockout mice. J Exp Med 1994; 180:43–51.
97. Korenaga M, Watanabe N, Tada I. Effects of anti-IgE monoclonal antibody on a primary infection of *Strongyloides ratti* in mice. Parasitol Res 1991; 77:362–363.

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20

The Rise of Antibodies as Therapeutics

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I. Introduction

Twenty-five years after their advent, monoclonal antibodies (MAbs) have emerged as an important and rapidly expanding new drug class for the treatment of severe human diseases (1–3). Ten antibodies have been approved in the United States for diverse clinical indications: cancer, transplant rejection, Crohn's disease, respiratory syncytial virus (RSV) prophylaxis, and as an anti-thrombotic (Table 1). An additional 70 or so antibody therapeutics are in clinical trials in the United States including at least 46 that have progressed to phase I/II or farther (Table 2). This chapter focuses on recent developments with antibody therapeutics from a biotechnology perspective.

II. Antibody Structure and Function

A. IgG Architecture

By far the most important class of antibody therapeutics is immunoglobulin G (IgG) and derived fragments, with only a very few IgM and IgA molecules currently in clinical development. IgGs are tetrameric molecules (~150 kDa) Y-

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Table 1 Antibodies Approved for Therapeutic Use in the United States

Product, antibody name	Technology	Antibody format	Antigen	Indication	Company	Worldwide 2000 sales[a] ($M[3])
Orthoclone OKT3, muromonab-CD3	Murine	IgG_{2a}	CD3	Prevention of acute kidney, heart, and liver transplant rejection	Ortho Biotech	
ReoPro, abciximab	Chimeric	Fab	$gpII_bIII_a$	Prevention of platelet-mediated clots in coronary angioplasty	Centocor	418
Rituxan, rituximab	Chimeric	IgG_1	CD20	Non-Hodgkin's lymphoma	IDEC, Genentech	424
Simulect, basiliximab	Chimeric	IgG_1	CD25[b]	Prevention of acute kidney transplant rejection	Novartis	
Remicade, infliximab	Chimeric	IgG_1	TNFα	Crohn's disease	Centocor	57
Zenapax, daclizumab	Humanized	IgG_1	CD25[b]	Prevention of acute kidney transplant rejection	Hoffman-La Roche	
Synagis, palivizumab	Humanized	IgG_1	RSV F glycoprotein	Prophylaxis of RSV in high-risk pediatric patients	MedImmune	427
Herceptin, trastuzumab	Humanized	IgG_1	*HER2/neu* antigen	Metastatic breast cancer overexpressing *HER2/neu*	Genentech	276
Mylotarg, gemtuzumab ozogamicin	Humanized, calecheamicin conjugate	IgG_4	CD33	CD33-positive acute myeloid leukemia	Wyeth Laboratories	
Campath, alemtuzumab	Humanized	IgG_1	CD52	B-cell chronic lymphocytic leukemia	Millennium & ILEX Partners, distributed by Berlex Laboratories	(approved 5/01)

[a] Sales data were obtained from company websites or by searching for ''*drugname* 2000 sales'' with search engine www.google.com.
[b] CD25 is the Tac subunit of the interleukin 2 receptor.
Source: Refs. 1,3,179,180, and company websites.

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Table 2 Antibody-Derived Therapeutic Agents in Advanced Clinical Trials in the United States[a]

Antibody name	Core technology	Antibody format	Enhancement technology	Target	Proposed use	Development stage	Sponsor
ABX-IL8	Human	IgG_2		IL-8	Psoriasis, chronic obstructive pulmonary disease	Phase II, Phase IIa	Abgenix
HuMax-CD4	Human	IgG_1		CD4	Rheumatoid arthritis, psoriasis	Phase II	Genmab A/S
ABX-EGF	Human	IgG_2		EGFR	Renal cancer	Phase II	Immunex, Abgenix
Xolair, omalizumab	Humanized	IgG_1		IgE	Allergic rhinitis, allergic asthma	BLA filed with FDA	Genentech, Novartis, Tanox Biosystems
Zamyl, SMART M195	Humanized	IgG_1		CD33	Acute myeloid leukemia	Phase III	Protein Design Labs
Xanelim, efalizumab	Humanized	IgG_1		CD11a	Psoriasis	Phase III	Genentech, XOMA
Avastin, bevacizumab rhuMAb-VEGF	Humanized	IgG_1		VEGF	Metastatic non–small-cell lung, colorectal	Phase III	Genentech
LymphoCide, epratuzumab	Humanized	IgG		CD22	Non-Hodgkin's lymphoma	Phase III	Immunomedics
Remitogen, SMART 1D10	Humanized	IgG_1		HLA-DR	Non-Hodgkins lymphoma	Phase II	Protein Design Labs
Humicade, CDP571	Humanized	IgG_4		TNFα	Crohn's disease	Phase IIb	Celltech
MDX-33 (H22)	Humanized	IgG_1		CD64 (FcγRI)	Idiopathic thrombocytopenia purpura	Phase II	Aventis Behring, Medarex
SCH5570	Humanized	IgG		IL-5	Asthma	Phase II	Schering Plough, Celltech
CEA-Cide, labetuzumab	Humanized	IgG	^{90}Y	CEA	Colorectal cancer	Phase I/II	Immunomedics
Antegren natalizumab	Humanized	IgG		VLA-4 ($\alpha_4\beta_1$ integrin) and $\alpha_4\beta_7$ integrin	Multiple sclerosis, Crohn's disease	Phase III	Elan, Biogen

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Table 2 Continued

Antibody name	Core technology	Antibody format	Enhancement technology	Target	Proposed use	Development stage	Sponsor
MEDI-507, siplizumab	Humanized	IgG_1		CD2	Psoriasis, graft-versus-host disease	Phase II	MedImmune
HuMax-IL15	Human	IgG_1		IL-15	Rheumatoid arthritis	Phase I/II	Genmab A/S, Immunex
Adalimumab, D2E7	Human	?		TNFα	Rheumatoid arthritis	Phase II	Abbott Laboratories
LDP-02	Humanized	IgG		$\alpha_4\beta_7$ integrin	Crohn's disease Ulcerative colitis	Phase II Phase I/II	Millennium
Orthoclone OKT4A	Humanized	IgG		CD4	CD4-mediated autoimmune diseases, transplant rejection	Phase II	Ortho Biotech
YM-337	Humanized	IgG		Gp II_bIII_a	Cardiovascular disorders	Phase II	Yamanouchi, Protein Design Labs
Anti-IL-4	Humanized	IgG_1		IL-4	Asthma	Phase I/II	Protein Design Labs
h5G1.1	Humanized	IgG		C5	Membranous nephritis, rheumatoid arthritis	Phase II	Alexion
IDEC-131	Humanized	IgG		CD40 ligand	Immune thrombocytopenic purpura	Phase II	IDEC, Eisai
Hu-901	Humanized	IgG		IgE	Peanut-induced anaphylaxis	Phase I/II	Tanox
SMART anti-gamma interferon	Humanized	IgG_1		IFN-γ	Crohn's disease	Phase I/II	Protein Design Labs
Nuvion, SMART anti-CD3	Humanized	IgG_2		CD3	Psoriasis	Phase II	Protein Design Labs
SMART anti-L-selectin	Humanized	IgG_4		L-selectin	Trauma	Phase II	Protein Design Labs, licensed in Europe to Scil Biomedicals GmbH
MDX-447	Humanized × humanized	$F(ab')_2$	Bispecific	EGFR × CD64	Head, neck, brain, breast, and bladder cancers	Phase II	Immuno-Designed Molecules, Merck KGaA

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Primatized-IDEC-151, clenolix-imab	Primatized[b]	IgG_1		CD4	Rheumatoid arthritis	Phase II	IDEC
IDEC-114	Primatized[b]	?		CD80	Psoriasis	Phase II	IDEC
IMC-C225, centuximab	Chimeric	IgG		EGFR	Colorectal cancer	BLA filing in-progress	ImClone Systems, Bristol-Myers Squibb Co.
Cotara, 131I-chTNT-1/B	Chimeric	IgG_1	^{131}I	DNA, histones	Recurrent glioblastoma multiforme	Phase III	Peregrine Pharmaceuticals
OvaRex, B43.13	Murine	IgG_1	Antibody modification by photoactivation	CA125	Ovarian adenocarcinoma	Plan to file BLA by end of 2001	AltaRex
BrevaRex	Murine	?	Antibody modification by photoactivation	PEM	Multiple myeloma	Phase I/II	AltaRex
Bexxar, 131I tositumomab	Murine	IgG_{2a}	^{131}I	CD20	Non-Hodgkin's lymphoma	BLA filed with FDA	Corixa
Zevalin, IDEC-Y2B8, 90Y ibritumomab tiuxetan	Murine	IgG_1	^{90}Y	CD20	Non-Hodgkin's lymphoma	BLA filed with FDA	IDEC
BEC2	Murine	IgG		Anti-idiotype GD3 mimic	Small-cell lung cancer, malignant melanoma	Phase III Phase II	ImClone Systems, Merck KGaA
Theragyn, pemtumomab	Murine	IgG_1	^{90}Y	PEM	Ovarian cancer, gastric carcinoma	Phase III Phase II	Antisoma
Panorex, edrecolomab	Murine	IgG_{2a}		EpCam	Colorectal cancer	Phase III	GlaxoSmithKline, Centocor,
Segard, afelimomab	Murine	$F(ab')_2$		TNF-α	Septic shock	Phase III	Abbott Laboratories
Enlimomab, BIRR-1	Murine	IgG_{2a}		ICAM-1	Stroke	Phase II/III	Boehringer Ingelheim
ABX-CBL	Murine	IgG		Activated B and T cells, monocytes	Graft-versus-host disease	Phase III	Abgenix
TriGem	Murine	IgG		GD2 mimic	Malignant melanoma	Phase II	Titan

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Table 2 Continued

Antibody name	Core technology	Antibody format	Enhancement technology	Target	Proposed use	Development stage	Sponsor
CeaVac	Murine	IgG	In combination with TriAb for NSCL (bivalent therapy)	CEA mimic	Colorectal cancer Non–small-cell lung cancer (bivalent therapy with TriAb)	Phase III Phase II	Titan
TriAb	Murine	IgG	In combination with CeaVac for NSCL (bivalent therapy)	HMFG mimic	Non–small-cell lung cancer (bivalent therapy with CeaVac) Breast cancer	Phase II Phase II	Titan
MDX-210	Murine × Humanized	$F(ab')_2$	Bispecific	*HER2/neu* × CD64 (FcγRI)	HER-positive ovarian cancer	Phase III	Medarex, Immuno-Designed Molecules
MDX-220	Murine × Humanized	$F(ab')_2$	Bispecific	TAG-72 × CD64	Lung, colon, prostate, ovarian, endometrial, pancreatic, gastric cancers	Phase I/II	Immuno-Designed Molecules
MDX-44	Humanized	IgG	Immunotoxin	CD64	Psoriasis	Phase I/II	Medarex
MDX-101	Human	IgG_1		CD152 (CTLA-4)	Prostate cancer, melanoma	Phase I/II	Medarex

[a] Clinical trials with antibodies that are already approved as drugs in the United States (Table 1) are not included.

[b] This primatized antibody contains variable domains from cynomolgus macaque (92% homology with human consensus sequence variable domains) and human IgG_1 constant domains (184,185).

BLA, Biologics License Application; TNFα, tumor necrosis factor alpha; CEA, carcino embryonic antigen; VLA-4, very late antigen 4; IFN-γ, interferon gamma; FDA, Federal Drug Administration; EGFR, epidermal growth factor receptor; CA125, cancer antigen 125; HMFG, human milk fat globule; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; PEM, polymorphic epithelial mucin; EpCam, epithelial adhesion molecule; CD152, CTLA-4, cytotoxic T lymphocyte antigen 4; VEGF, vascular endothelial growth factor.

Source: Refs. 1, 3, 179–185, company websites, www.BioSpace.com, and personal communications from Nilslonberg (Medarex Inc.) and Harold Keer (Titan Pharmaceuticals).

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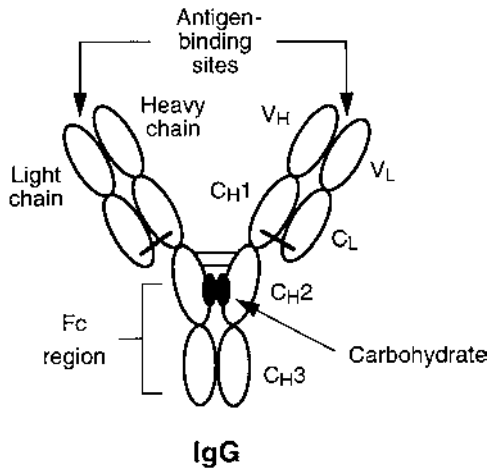
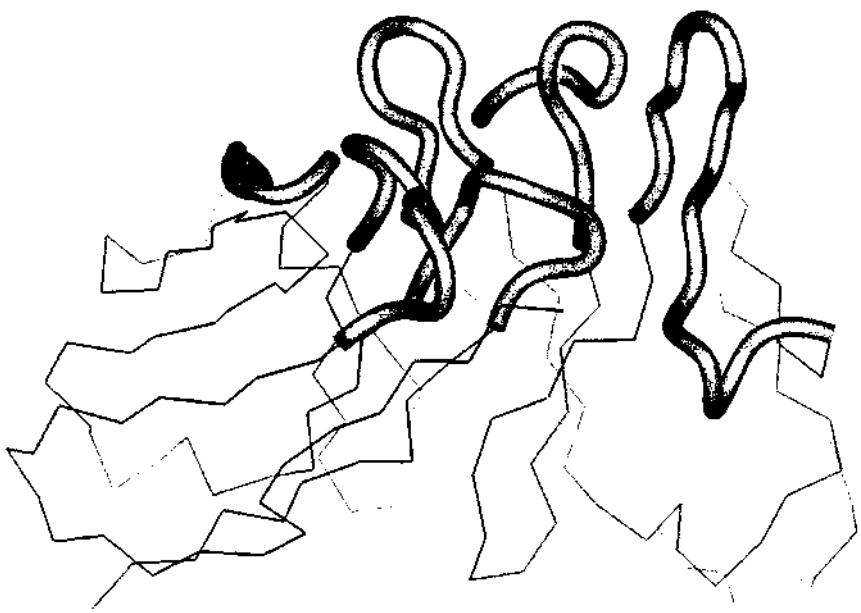


(a)



(b)

Figure 1 Immunoglobulin architecture. (a) Schematic representation of an IgG. A single disulfide bond links the constant domains of heavy and light chains (diagonal line), whereas the heavy chains are interconnected by one or more disulfide bonds in the hinge region located between C_H1 and C_H2 domains (horizontal lines). A disulfide bond present within each domain is omitted for clarity. (b) X-ray crystallographic structure of the Herceptin (huMAb4D5-8) (77) Fv fragment comprising V_L and V_H domains (178). The antigen-binding CDR residues are represented as tubes, whereas the remainder of the variable domains (FR residues) are shown as thin lines.

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shaped molecules that contain two antigen-binding sites. IgGs are comprised of two identical heavy chains plus two identical light chains (Fig. 1a). IgG heavy chains contain a variable domain, V_H, plus three constant domains, C_H1, C_H2, and C_H3, whereas light chains encompass a variable domain, V_L, plus a single constant domain, C_L. Antigen binding is mediated primarily by six loops known as complementarity-determining regions (CDRs), three of which are contributed by each of the V_H and V_L domains (Fig. 1b). The remainder of the variable domains, known as framework regions (FRs), serves mainly as a scaffold to support the CDRs. In some cases, one or a few FR residues may directly contact bound antigen (4).

B. Fc-Mediated Functions of IgG

Human IgGs are represented by four highly homologous heavy-chain isotypes: IgG_1, IgG_2, IgG_3, and IgG_4 and two light-chain isotypes, κ and λ. The heavy-chain isotypes differ in their constant domains as well as in their flexible hinge region between C_H1 and C_H2 domains. Two human IgG isotypes, namely IgG_1 and IgG_3, have the potential to support cytotoxic secondary immune functions: complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) by virtue of their Fc regions (C_H2 and C_H3 domains) (5,6). Once a suitable IgG has bound to a target cell, it may potentially mediate CDC or ADCC. Initiation of CDC involves binding of the IgG Fc region to the complement component, C1q, which in turn triggers complement activation. This culminates in the assembly of a lytic complex that disrupts the cell membrane of the target cell. ADCC begins with the interaction of an IgG Fc region with one of several types of Fcγ receptors on immune effector cells such as neutrophils and macrophage. This interaction unleashes the killing machinery of the effector cell upon the hapless target cell. In addition to its role in ADCC and CDC, the Fc region is also important in maintaining the long plasma persistence of IgG, apparently by virtue of its interaction with the receptor, FcRn (7–9).

III. Problems That Have Beset Antibody Therapeutics

Numerous difficulties hampering the development of antibody therapeutics have long been recognized and extensively reviewed (6,10). These problems with MAb drugs are related to the choice of antibody, antigen, and target tissue but also include pharmaceutical and clinical issues. The most important problems are recapitulated here, as understanding and addressing them has been key to the recent rise of antibodies as therapeutics.

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A. Antibody-Related Issues

Arguably the single most significant problem that has stymied the therapeutic use of MAb has been the human antimouse antibody (HAMA) response elicited by these foreign proteins. A HAMA response occurs in a high proportion of patients, particularly following repeat MAb administration (11–14). The HAMA response may compromise MAb efficacy by direct neutralization, acceleration of clearance, prevention of multiple dosing, and by causing serum sickness. Nevertheless, HAMA responses may occasionally be beneficial, as suggested by the correlation between anti-idiotypic (anti-CDR and anti-anti-CDR) responses and favorable clinical outcome (15–18). The HAMA problem has been greatly diminished by the use of chimeric, humanized, and human antibodies (Sec. IV) as well as by antibody fragments (Sec. V).

Many murine MAbs, depending upon their heavy chain isotype, are very inefficient in their ability to support ADCC and CDC. Effector functions are readily recruited into murine MAb by chimerization or humanization (Sec. IV). Indeed, 7 of 10 approved MAb drugs utilize human IgG_1 constant domains and are thus potentially capable of mediating ADCC and CDC (Table 1).

B. Antigen-Related Issues

Cell surface antigens are seldom completely restricted to the target tissue. Consequently, antibody therapeutics may bind to antigen-positive normal tissue in addition to the target. This can sometimes compromise efficacy and cause toxicity. For example, an antiadenocarcinoma MAb that cross-reacts with cells lining the gastrointestinal tract has been associated with a high incidence of diarrhea, nausea, vomiting, abdominal pain, and even bowel mucosal damage (19). Toxicity to antigen-expressing normal tissue may be exacerbated when MAbs are armed to enhance their potency (Sec. VII. A). For example, immunotoxins that cross-react with neural tissue have been associated with specific pain syndromes (20,21). Judicious selection of the target antigen is a crucial aspect of the development of antibody therapeutics that is beyond the scope of this review.

In the case of cell surface antigens, secretion or shedding into the circulation may abrogate their therapeutic benefit by either accelerating MAb clearance or by acting as sink for the MAb (22). This issue should be taken into account in the selection of a target antigen.

C. Problems in Tumor Targeting

A major difficulty with tumor immunotherapy is the meager quantity of MAb that localizes to a tumor—typically $\leq 0.01\%$ of the injected dose per gram of tumor—combined with the often heterogeneous distribution of MAb within the

tumor (6,10). Inefficient and heterogeneous accumulation of MAb within tumors reflects multiple physiological barriers (23,24) as well as antigen-related issues (6,10). The chaotic vasculature and lack of lymphatic drainage from solid tumors may lead to elevated interstitial pressure that subsequently limits extravasation and intratumoral diffusion of MAbs (25,26). This problem is heightened by the large size of IgGs and can impede their penetration into solid or bulky tumors (27–29). Variations in tumor vascularity may limit diffusion of therapeutic antibodies to well-perfused areas of tumor (30).

Antigen-related problems include the downregulation of antigen expression after MAb binding, antigen mutation, heterogeneous expression, and the internalization of the antigen-antibody complex (6,10,31,32). The relationship between antigen-binding affinity and the ability of an antibody to penetrate a tumor is a complex one that is influenced by antigen density. High density of target antigen and/or high antigen-binding affinity can sometimes lead to MAbs being trapped by perivascular tumor cells, thereby hindering MAb penetration into the tumor (24,33). Conversely, increasing the antigen-binding affinity of a MAb (34) or scFv (35) has also been reported to enhance tumor retention, as has upregulation of antigen expression (36).

Several promising strategies have been explored to address the problem of inefficient MAb accrual in bulky tumors. First, as exemplified by *Panorex* (Sec. IV.A), MAb may be used to target more accessible minimal residual disease and micrometastases instead of bulky disease. Second, antibody fragments (Sec. V) are better able to penetrate solid tumors than the much larger IgG, albeit at the expense of greatly reduced plasma permanence time and total accrual in the tumor. Third, targeting the highly accessible tumor vasculature is an attractive alternative to the comparatively inaccessible tumor (24,37,38).

Damage to the vasculature may cause widespread tumor cell death as each capillary nourishes many tumor cells. Moreover, the vascular endothelial cells unlike the tumor cells are not transformed and thus seem less likely to become resistant to MAb therapy through mutation. The concept of targeting the vasculature supplying the tumor, rather than the tumor itself, has been convincingly demonstrated in tumor xenograft studies in mice (37,38). A bispecific antibody was used to target tissue factor to an artificial marker of angiogenesis to induce intravascular vascular thrombosis within the tumor, resulting in complete tumor regression in 38% of mice (38). Recently Neri and colleagues extended this approach by targeting tissue factor to the ED-B domain of fibronectin, a natural marker of angiogenesis present in many solid tumors and undetectable in most normal vessels and tissues. Up to 30% of the mice treated had their tumors eradicated with no obvious side effects (39), encouraging further preclinical development of this strategy.

Residual tumor cells can proliferate following therapeutic MAb treatment and those lacking the target antigen, or even just the antibody-binding epitope,

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will be resistant to further MAb treatment (40–42). This mechanism for tumor resistance to MAb therapy has encouraged the development of antibody enhancement technologies such as radioimmunotherapy (RIT) and targeted prodrug therapy, which permit the killing of antigen negative bystander cells (Secs. VII.A and VII.C).

D. Pharmaceutically Related Issues

Antibodies, like other protein pharmaceuticals, are not orally bioavailable because of protein degradation in the gastrointestinal tract and the low penetrability of the gastrointestinal epithelium. Thus, antibody therapeutics are limited to parenteral routes of administration. Antibody therapeutics are typically formulated to yield shelf lives comparable to those of traditional pharmaceuticals. However, antibodies, unlike traditional pharmaceuticals, are susceptible to multiple mechanisms of degradation including loss of their three-dimensional structure through denaturation (43,44). Denaturation results in loss of activity and can occur during storage under excessively high or low temperatures, freezing and thawing of solutions, or excessive agitation of the solution either during reconstitution or handling (44,45). Thus, antibodies require special care with their storage, preparation, and handling (Table 3) and require close adherence to manufacturers' prescribing information. Failure to do so may have deleterious effects upon the stability and bioactivity of antibody therapeutics.

E. Clinical Issues

An infusion-associated symptom complex can occur with the infusion of MAbs that bind to circulating cells, especially B or T lymphocytes, granulocytes, or leukemia cells (19). The typical symptom complex includes fever, chills, diaphoresis, nausea, dyspnea, and hypotension. Studies with radiolabeled cells have

Table 3 General Handling and Stability Issues for Antibody Therapeutics

Limited to parenteral administration
Incompatible with preservatives, precludes multidose vials, limits aliquot dosing and extended infusion periods
Require reconstitution and/or dilution prior to administration
Limited aqueous solubility precludes excessive dilution
Freezing the reconstituted solutions is deleterious
Vigorous agitation or shaking may lead to denaturation
Can be adsorbed onto materials used in intravenous tubing, filters, bags, and syringes
Limited stability at room temperature
Should not be mixed with other intravenous solutions

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shown that once an antibody binds to circulating cells, they are rapidly removed by macrophages in the lungs, liver, and spleen. Dyspnea and hypotension may result when large numbers of cells are removed via the lungs. Many symptoms that are related to the removal of circulating target cells are likely secondary to the release of cytokines such as interleukins and interferons. Initial infusions of *OKT3* and Rituxan have been associated with acute clinical syndromes termed cytokine release (45) and tumor lysis syndromes, respectively (46).

Precipitate toxicity can result from the rapid infusion of MAbs that elicit cellular activation and cytokine release. Prolonged continuous infusion may be necessary for therapeutic MAbs in order to maintain downregulation of receptors or blocking of receptors (19). MAb administration to patients with circulating antigen results in immune complex formation that can lead to serum sickness and end-organ damage (14). Serum sickness reflects a vasculitic process initiated upon deposition of circulating immune complexes in blood vessel walls (47). Complement activation and IgE-mediated histamine release are among the factors that produce characteristics of serum sickness such as fever, arthralgia, palpable purpura, urticaria, and lymphadenopathy (48). The onset of serum sickness may correlate with the development of a HAMA response (14) and can occur within hours of MAb administration in sensitized individuals.

MAb therapy may also cause acute hypersensitivity reactions that are primarily mediated by IgE. Anaphylactic reactions following administration of proteins can occur. Medications for the treatment of severe hypersensitivity should be available for immediate use. Additionally, antibodies of the IgG or IgM class may develop, resulting in delayed hypersensitivity reactions or neutralization of the effects of the therapeutic MAbs (49). Autoimmunity may also develop during the course of MAb therapy. For example, Remicade therapy has been associated with anti-DNA antibodies and lupus-like symptoms (Centocor, Inc., Remicade prescribing information, 2000). Other safety-related clinical aspects of MAb therapy include consideration of secondary exposure of a fetus or nursing child. Antibodies can be excreted in breast milk, and IgG molecules, which make up the majority of the therapeutic MAbs, are known to cross the placental barrier.

IV. Core Antibody Technologies

A few core antibody technologies, reviewed in Ref. 50, have been crucially important to the development of all marketed antibody therapeutics (Fig. 2) as well as to virtually all antibody therapeutics in clinical development. These core antibody technologies are reviewed in this section: hybridoma, chimerization, humanization, and human antibodies through phage libraries or transgenic mice. Approximately 11 years elapsed between the advent of hybridoma, chimerization,

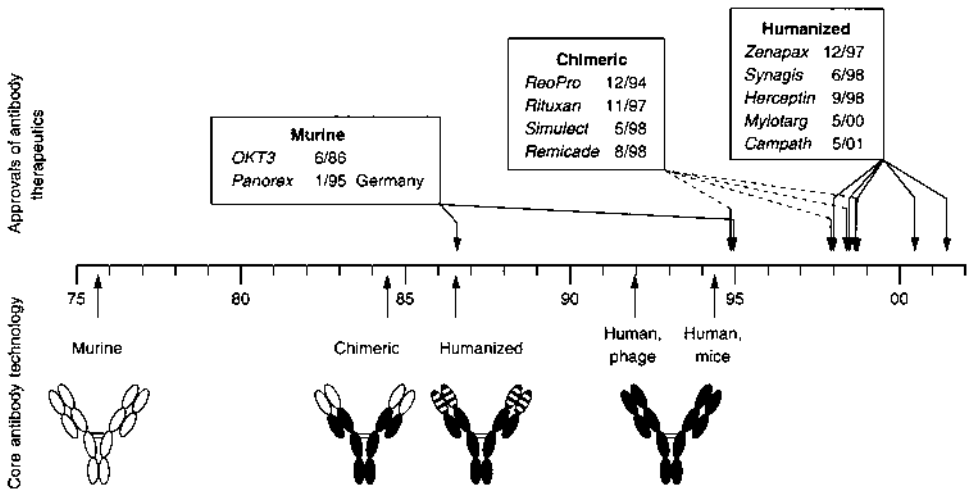

Figure 2 Comparison of the timelines for the emergence of core antibody technologies versus the regulatory approval of antibody therapeutics using these technologies. Murine and human sequences are represented by open and filled symbols, respectively.

and humanization and the approval of the first antibody drugs using each of these technologies. This technology cycle time reflects the time-consuming, complex, and expensive drug-development process. Similar cycle times are anticipated for the emerging technologies for creating human antibodies.

A. Murine Monoclonal Antibodies

MAbs came into being in 1975 with the ''Nobelogenic'' invention of hybridoma technology by Köhler and Milstein (51). Unfortunately, the high therapeutic expectations of murine MAbs have been stymied by their lack of efficacy in most clinical trials. A major contributory factor to the poor clinical performance of many murine MAbs has been the HAMA response they induce (Sec. III. A). Nevertheless, one murine MAb has been approved as a drug in the United States, namely the immunosuppressive anti-CD3 antibody, OKT3, for the acute treatment of organ rejection following transplantation (Table 1). The murine MAb, 17-1A, binds to epithelial cell adhesion molecule (EpCam) (52) and is in phase III clinical trials in the United States for the treatment of Dukes' C colorectal cancer (Table 2). MAb 17-1A has already been approved, as Panorex, in Germany for the adjuvant therapy of colorectal cancer (53,54). Treatment with Panorex decreased the overall mortality rate by 32% and the recurrence rate by 23% (54).

Panorex was more efficacious in the treatment of minimal residual disease and micrometastases (53,54) than in bulky metastatic disease (55). This finding is widely anticipated as a paradigm that will be broadly applicable to other antitumor antibodies.

B. Chimeric Antibodies

A partial solution to the MAb immunogenicity problem came in 1984 with the advent of antibody chimerization (56,57). Chimeric antibodies are created by coupling the antigen-binding variable domains of a murine MAb to human constant domains (Fig. 2). The isotype of the human heavy chains may be chosen (see Sec. II) so that chimeric antibodies can be optionally conferred with cytotoxic secondary immune functions (58). Chimeric antibodies are still ~30% rodent in sequence and in some cases elicit a human antichimeric antibody (HACA) response that is typically less frequent, slower to develop, and lower in titer than the HAMA response observed with the corresponding murine parent MAb (59,60). In the case of the chimeric anti-CD20 antibody, Rituxan, only very infrequent and minor HACA responses have been observed (61,62) Rituxan was the first antibody to be approved in the United States for cancer treatment following response rates of 48% in non-Hodgkin's lymphoma (61,62). For some murine MAbs (59), but not others (60), chimerization substantially increases the plasma persistence in patients.

Four chimeric antibodies including Rituxan have been approved as drugs in the United States, starting in 1994 with ReoPro, a Fab fragment that binds to the integrin, II_bIII_a (Table 1). ReoPro is one of the most commercially successful antibody therapeutics to date with worldwide sales of $418 M in 2000 (Table 1). ReoPro provides a clinical proof of concept that inhibition of platelet-mediated blood clotting can reduce the ischemic complications of coronary angioplasty and atherectomy, albeit at the cost of an increased risk of bleeding (63) ReoPro acts as an antagonist in blocking the interaction between the platelet glycoprotein II_bIII_a and ligands containing the RGD motif. A small molecule drug can readily mimic such a mechanism of action and has the potential advantage over ReoPro in being cheaper to manufacture and, in some cases, orally bioavailable. Indeed, two injectable II_bIII_a antagonists have already been approved as drugs, namely the heptapeptide Integrilin and Aggrastat, a protein from the venom of the saw-scaled viper. Additionally, three orally bioavailable II_bIII_a antagonists progressed as far as phase III clinical trials. However, small molecule II_bIII_a antagonists have not matched the desirable long plasma persistence of ReoPro, and it remains to be seen if any of them will prove to be effective competitors. Nevertheless, the twin incentives of reduced cost and oral bioavailability will likely drive the development of small molecules as competitive or second-generation products for other antibody therapeutics that function as antagonists.

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C. Humanized Antibodies

The next major advance in therapeutic antibody technologies was the invention of ''humanization'' in the late 1980s—an outstanding feat of protein engineering (64–66). The simplest form of humanization involves transplanting the antigen-binding CDR loops, from a rodent MAb into a human IgG (Fig. 2). In most cases one or more additional FR residues are required to confer high antigen-binding affinity. Subsequently, several alternative humanization strategies have been developed, (reviewed in Ref. 50). Humanized antibodies contain as few as 5% rodent residues and are anticipated to be much less immunogenic than their rodent parent antibodies and somewhat less immunogenic than corresponding chimeric antibodies. Broadly speaking, humanized antibodies have lived up to expectation in the clinic in that upon repeat administration they have elicited either no detectable (67–70) or minor human antihuman antibody (HAMA) responses including anti-idiotype antibodies (71–73).

Five humanized antibodies have been approved as drugs in the United States beginning in 1997 with the anti-CD25 antibody, Zenapax, for the prevention of acute kidney rejection (74) (Table 1). The humanized anti-RSV antibody Synagis has been approved for the prophylactic treatment of high-risk pediatric patients (75) (Table 1). Synagis is one of the most commercially successful antibody therapeutics, with sales of $427 M in 2000 (Table 1). The humanized anti-CD33 antibody calicheamicin conjugate Mylotarg has been approved for the treatment of acute myeloblastic leukemia. Mylotarg has the distinction of being the first armed antibody to be approved for human therapy (see Sec. VII.A). The humanized antibody anti-CD52 Campath has proved efficacious in the treatment of B-cell chronic lymphocytic leukemia and was approved for this indication in 2001. Campath was the first therapeutically relevant antibody to be humanized (65) and the first to reach the clinic in 1988 (76). The protracted clinical development of Campath reflects, at least in part, its lackluster performance in some early clinical trials including toxicity problems, not to mention changes in ownership of this antibody.

The humanized anti-HER2 antibody huMAb4D5-8 (77), now marketed as Herceptin, was approved for the treatment of metastatic breast cancer in patients whose tumors overexpress the *HER2/neu* antigen. The antitumor response rates in a phase III trial were 50% for Herceptin in combination with chemotherapy as compared to 32% for chemotherapy alone (78). Herceptin has also been a significant commercial success with sales of $ 276 M in 2000 (Table 1). Both Herceptin (67,69,78) and also Rituxan (61,62) are generally well tolerated by cancer patients and have given rise to toxicities that are commonly much less severe than those from conventional chemotherapy. Numerous additional humanized antibodies are currently in clinical trials in the United States, including more than 20 that have reached at least phase I/II trials (1,3) (Table 2).

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D. Human Antibodies Through Phage Display Libraries

The 1990s have witnessed the emergence of vast antibody phage libraries for the very rapid identification of human antibodies (reviewed in Ref. 50). A prevalent version of this technology involves cloning of large repertoires of V_H and V_L genes from naïve human donors and their display as single-chain (sc) Fv or Fab fragments (see Sec. IV and Fig. 4 for fragment description) on the surface of filamentous phage (Fig. 3). Application of antibody phage libraries entails the identification of antigen-specific phage by in vitro binding selection—''biopanning''—with the target antigen. The first antibody phage library ($>10^7$ clones) demonstrated the concept that moderate affinity (K_d = 100–500 nM) human antibodies could be obtained to target antigens (79). The subsequent construction of much larger phage libraries ($\geq 10^9$ clones) has allowed the rapid and routine isolation of high-affinity human antibodies (K_d = 0.2–70 nM) to a wide array of antigens (80–85).

For some therapeutic applications it may be necessary to increase the antigen-binding affinity of phage-derived antibodies or tailor their specificity. The phage format is particularly well suited to these tasks as it allows mutagenesis of antigen-binding residues and rapid selection for altered binding properties. For example, affinity maturation from the nanomolar to the picomolar range has been demonstrated for anti-gp120 (86) and anti-$p185^{HER2}$ antibody fragments (87). Antibody fragments identified using phage libraries are readily recast as IgG or a multitude of alternative formats (Sec. V) in preparation for clinical investigation.

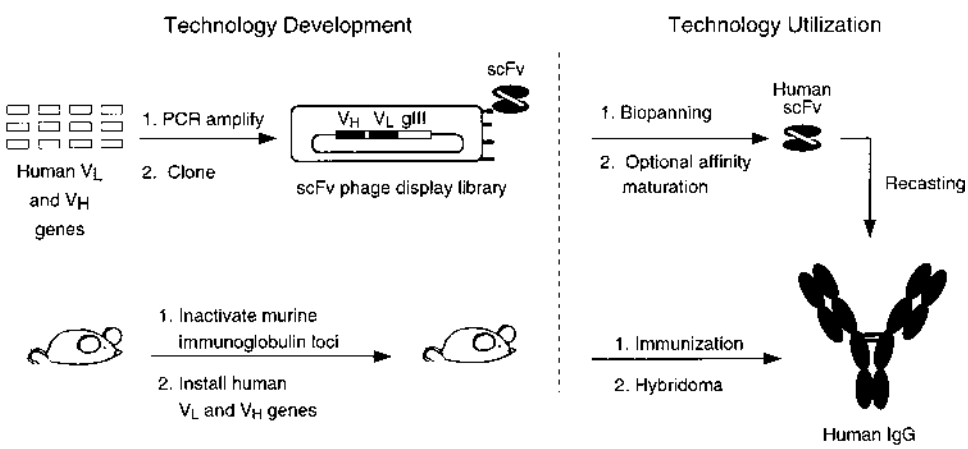

Figure 3 Antibody phage library and transgenic mouse technologies for preparing human antibodies.

At least four human IgGs derived from phage libraries are currently in clinical trials in Europe including antibodies that target tumor necrosis factor α (TNF-α, phase III for rheumatoid arthritis) and transforming growth factor β_2 (TGF-β_2, phase II for treatment of scarring following glaucoma surgery), interleukin 12 (IL-12, phase II for autoimmune diseases), and TGF-β_1 (phase I for fibrosis and scarring) (2) (www.cambridgeantibody.com).

Antibody library technology continues to evolve with improvements in the quality of libraries, more robust expression of identified antibody fragments in *Escherichia coli* and easier recasting into other antibody formats. Ribosome display is an emerging in vitro selection technology that has broad applicability including to antibody (82,88). Ribosome display may eventually supersede phage display in light of its potential advantages: faster, larger libraries, and more ready evolution of identified antibodies (89).

E. Human Antibodies from Transgenic Mice

High-affinity human antibodies have also been obtained from transgenic mice containing human antibody genes and disrupted endogenous immunoglobulin loci (Fig. 3). Immunization leads to the production of human antibodies that can then be recovered using standard hybridoma technology (50,90). The multimegabase size of the human immunoglobulin loci combined with the complex segmented gene structure were formidable obstacles that were surmounted in the development of transgenic mice making human antibodies. Mice containing only a limited selection of human immunoglobulin genes are still capable of yielding high-affinity antibodies that are antigen specific (91). Mice containing a larger, although still incomplete, set of human variable genes closely recapitulate IgG production seen in humans, including gene rearrangement, diverse gene usage, and somatic hypermutation. These mice have yielded high-affinity human IgG_2 antibodies to multiple antigens, including human proteins (92).

Ongoing improvements to this technology include the development of mice generating human IgG_1 antibodies to provide support for secondary immune functions. In addition, expanding the variable gene repertoire is anticipated to facilitate the generation of even larger panels of high-affinity antibodies against diverse antigens. For example, the gene repertoire has been expanded by inclusion of the entire immunoglobulin heavy chain and κ light chain loci (93) and alternatively by the addition of genes for λ light chains (94).

At least three human antibodies derived from transgenic mice are in clinical trials as of mid-2001, including antibodies that target interleukin 8 (IL-8, phase IIa for rheumatoid arthritis and phase IIb for psoriasis), CD4 (phase II for rheumatoid arthritis), and epidermal growth factor receptor (EGFR, phase I for EGF-dependent cancers). The anti-EGFR antibody was found to eradicate very large established tumors in some preclinical xenograft models (95).

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F. Choice of Core Antibody Technology

Humanized and human antibodies are the currently preferred core technologies for developing antibody therapeutics. Humanization may be the favored strategy when a well-characterized murine MAb is available. In contrast, for a newly identified antigen where no MAbs are available, direct production of human antibodies using mice or phage are preferred. Many more human antibodies from phage and mice are anticipated to enter clinical trials as these technologies become more widely available, e.g., through corporate partnerships. The choice between these preferred core antibody technologies will likely depend upon their availability and local expertise as well as commercial considerations such as patents and royalty payments.

V. Formats for Antibody Therapeutics

In addition to the naturally occurring IgG format, numerous alternative recombinant antibody fragments have been constructed by capitalizing upon the modular architecture of IgG (96) (Fig. 4). Reformatting makes it feasible to customize an antibody so that properties such as pharmacokinetics and the ability to support cytotoxic secondary immune functions are matched to the target indication. In addition, the format of an antibody therapeutic will impact the choice of suitable production organisms (Sec. VI). In this section the expanding range of antibody formats is explored together with factors that impact the choice of format in developing antibody therapeutics.

A. Alternative Antibody Formats

The earliest reformatting of antibodies involved limited proteolysis of MAb to yield either monovalent Fab fragments or bivalent $F(ab')_2$ fragments depending upon the protease used and the isotype of the MAb (97). Currently, Fab and $F(ab')_2$ fragments are more conveniently derived by recombinant expression in *E. coli* (98).

The most widely used antibody fragment format is the scFv. The scFv comprises the antigen-binding V_L and V_H domains of an IgG connected with a short linker (15–20 residues) to prevent domain dissociation (99,100). The scFv has been the predominant building block for the design of more complex fragments. For example, bivalent and also bispecific fragments have been constructed by joining scFv by means of an additional linker [$(scFv)_2$ (101,102)], directed chemical coupling [$(scFv')_2$ (103)] or a protein dimerization motif [minibody (104); miniantibody (105,106)]. Alternative multimerization motifs have been used to create miniantibodies that are tetravalent (107) or bispecific and bivalent for each binding specificity (108).

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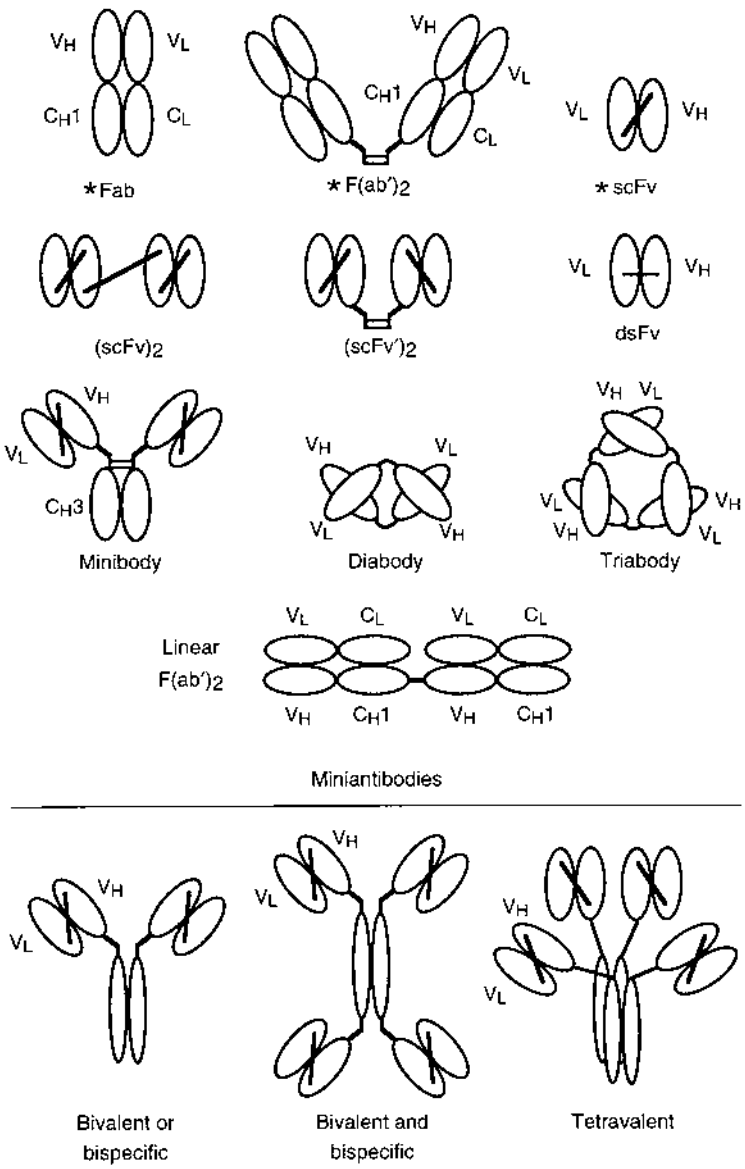

Figure 4 Alternative formats for recombinant antibody fragments. Formats that have been tested clinically are highlighted (*). A linker connecting the carboxy terminus of V_L to the amino terminus of V_H is indicated as a bold line for all scFv-related formats. The alternative topology of connecting the carboxy terminus of V_H to the amino terminus of V_L has also been used successfully in most cases but is omitted for simplicity. DsFv, disulfide-stabilized Fv fragment.

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Diabodies are small bivalent or bispecific fragments resulting from the dimerization of scFv fragments (109). Diabody formation relies upon the use of short linkers (5–10 residues) to allow interchain pairing of variable domains while preventing intrachain pairing (109) (Fig. 4). Diabodies are one of the most promising routes to bispecific antibodies fragments in that they can, in one case at least, be secreted at very high titer from *E. coli* (110) (Sec. VI B). The clinical potential of diabodies has been enhanced by conferring to them the ability to support ADCC and CDC and by increasing their terminal half-life in vivo approximately sixfold to 10 hours. This was accomplished with a bispecific diabody in which one arm binds to plasma IgG (111). An alternative strategy to enable a bispecific diabody to support CDC is by targeting one specificity to the complement component, C1q (112).

One marketed antibody therapeutic, ReoPro, is in Fab rather than IgG format (Table 1). In addition, one $F(ab')_2$ fragment derived by limited proteolysis of murine MAb is currently in phase III therapy trials (Table 2) (1,3). Thus far, recombinant scFv (113) and $F(ab')_2$ (114) fragments have been tested in the clinic and more recently PEGylated versions of Fab′ and $F(ab')_2$ fragments (see below).

B. Choice of Antibody Format

One of the attractions of antibodies as therapeutics is that several of their properties are broadly predictable and readily varied. This makes it feasible to customize the format of an antibody to match the target clinical indication. This involves a series of decisions that are guided ideally by direct comparison of alternative formats in preclinical experiments as well as by preclinical and clinical experience with other antibodies. Key decisions include the following: (1) target pharmacokinetic properties, (2) desirability of secondary immune functions, (3) desired valency, (4) desired affinity for the target antigen, and (5) pharmacoeconomics (Table 4). Several examples utilizing different antibody formats are provided below to illustrate this complex decision-making process.

Herceptin is a humanized IgG_1 with high binding affinity for the *HER2/neu* antigen (K_d = 0.1 nM) (77). The IgG format was chosen for the intended chronic treatment of metastatic breast cancer, since a long half-life in patients was predicted to favor antitumor activity. A mean terminal half-life of 9–11 days was observed in two phase II trials with Herceptin, although this was reduced to 2–4 days in Herceptin patients with elevated levels ($\geq$0.5 μg/mL) of shed antigen (67,69). The IgG_1 isotype was selected as the ability to support ADCC was found to be a potent antitumor mechanism in preclinical experiments in vitro (77,115). Subsequently it has been shown that functional Fc receptors are critical to the potent antitumor activity of Herceptin (and also Rituxan) in tumor xenograft studies in vivo (116). A bivalent rather than monovalent format was used since the former favors cytostatic activity in vitro (117). The impacts of affinity

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Table 4 Factors Affecting Choice of Antibody Format

1.	Pharmacokinetics
	Plasma persistence: IgG $\gg$ F(ab′)$_2$ > Fab > scFv
	Antigen influence: may decrease or increase plasma persistence
2.	ADCC and CDC
	When desired: IgG$_1$, IgG$_3$
	When *not* desired: IgG$_2$, IgG$_4$, antibody fragments
3.	Valency
	Monovalent: Fab, scFv
	Bivalent: IgG, F(ab′)$_2$, diabody[a]
4.	Affinity
	Readily accessible range: 10 pM–100 nM
	Optimal: unknown
5.	Pharmacoeconomics[b]
	Production costs: mammalian cell culture > *E. coli* > transgenic plants/animals

[a] See Figure 4 for additional options.
[b] Note that the relative cost of goods of antibody production in different host organisms is very significantly impacted by the scale of production (Sec. VI).

on tumor localization and antitumor activity are unknown, so the highest affinity humanized variant was chosen (77).

A humanized anti-CD 18 F(ab′)$_2$ fragment was designed to block neutrophil-mediated interactions in acute indications and progressed to phase II clinical trials for hemorrhagic shock (114). A fragment was chosen over an IgG as a short half-life was desired to allow for more ready reversal of drug treatment. The F(ab′)$_2$ format was adopted as this was the smallest fragment that demonstrated comparable efficacy to IgG in preclinical animal experiments. *E. coli* was selected as a production organism as this was significantly cheaper than the alternative of mammalian cell culture (Secs. VI. A and B).

The scFv fragment, MFE-23, binds to carcinoembryonic antigen (CEA) and has been used to image tumors in patients as soon as 1–4 hours following treatment (113). The scFv format was chosen because such small fragments (~27 kDa) penetrate tumors more readily than do larger fragments and IgG in xenograft models (118). In addition, the small size of scFv fragments results in rapid clearance from plasma and consequently high tumor: nontumor ratios within a few hours of administration, as shown in tumor xenograft studies (119–121). A disadvantage of the rapid clearance of scFv fragments is that the total accumulation in the tumor is much less than for corresponding IgG and F(ab′)$_2$ fragments (118). Thus scFv fragments are well suited to radioimmunoimaging, but much less so for RIT where efficient accrual in the tumor is paramount (6,122).

IV. Production of Antibody Drugs

Reducing the high cost of producing antibody therapeutics is an important problem that must be addressed so that the benefits of these drugs may be extended to more of humanity. Numerous host systems have been explored for the large-scale production of antibodies (123,124), including several with near-term commercial potential that are discussed here.

A. Mammalian Cell Culture

All marketed antibody drugs are produced in hybridoma, Chinese hamster ovary, or NSO cells at titers of up to ~2 g/L. The primary drawback of these mammalian cell culture methods is that they can be a major bottleneck in the development of antibody drugs in light of the worldwide shortage of mammalian production capacity (125). A secondary consideration is the high cost of antibodies produced by mammalian cell culture, estimated as $300–1000/g on a 100 kg/yr scale (124). This may compromise the commercial viability of antibodies for the treatment of chronic indications requiring multigram total doses.

B. *E. coli*

Secretion from *E. coli* is the most widely utilized method for the production of recombinant antibody fragments in the research laboratory. In contrast, limited proteolysis of IgG has been the most prevalent source of antibody fragments for clinical trials for imaging and therapy (Table 2) (1,3). Expression in *E. coli* will likely supersede mammalian production of antibody fragments for future clinical trials as it is much cheaper and may result in faster development timelines. Moreover, it has been possible to apply the well-established biotechnology paradigm of enhancing production titers in *E. coli* by culturing to high cell density in a fermentor. Indeed, gram per liter titers have been achieved for several antibody fragments including Fab′ (126), bispecific diabody (110), and miniantibody (127).

These successes notwithstanding, a significant limitation of antibody fragment expression in *E. coli* is the variability in titer between different antibodies. Titers are highly dependent upon antibody primary sequence and culturing conditions and usually to a lesser extent upon the expression vector and *E. coli* host strain (98). Nevertheless, several methods to enhance expression titers of antibody fragments have been developed (96). Although functional IgG molecules have been secreted from *E. coli* (P. Carter, unpublished data), the low titers (≤10 mg/L) and inability of *E. coli* to perform glycosylation diminishes the commercial potential of this finding.

Unfortunately, the rapid clearance of Fab and even $F(ab')_2$ fragments renders them unsuitable for many chronic clinical indications that require maintenance of serum antibody concentrations over extended periods of time. A major

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breakthrough in this area has been the dramatic prolongation of in vivo half-lives of Fab′ (128) and F(ab′)$_2$ (A. Chapman, personal communication) fragments by their site-specific modification with polyethylene glycol (PEG). Indeed, the pharmacokinetic properties of an IgG in cynomolgus monkeys was virtually recapitulated by a judiciously PEGylated Fab′ fragment (128). This technology seems likely to significantly extend the clinical potential of antibody fragments such as those expressed in *E. coli*.

The first reported clinical trial using an *E. coli*–derived antibody fragment was a radioimmunoimaging study using a scFv fragment derived from a combinatorial library (113). An *E. coli*–derived F(ab′)$_2$ has also been evaluated in clinical trials (114), whereas clinical evaluation of PEGylated Fab′ and F(ab′)$_2$ fragments is just beginning (A. Chapman, personal communication).

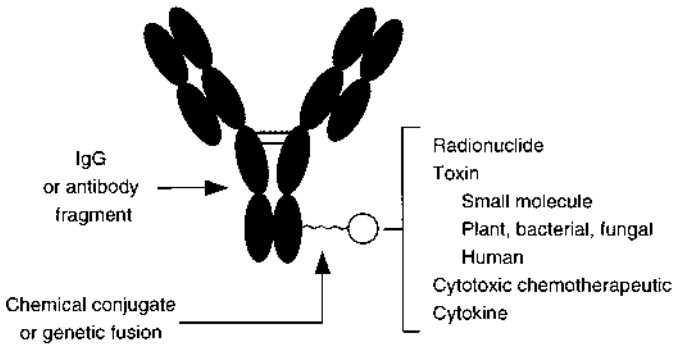
C. Transgenic Animals

The production of protein pharmaceuticals in the milk of transgenic animals is a promising technology that has yet to gain widespread acceptance and use. IgG molecules have been produced in the milk of transgenic goats at titers of up to 14 g/L (129). Goats are an attractive production method for producing ''very large'' but not ''small'' quantities of antibodies—costs estimated as $40/g at a 1000 kg/y scale but rising to $300/g on a 10 kg/y scale. One protein from transgenic animals, antithrombin III, is already undergoing clinical evaluation. A significant downside to the production of protein pharmaceuticals in transgenic animals is that animal breeding is likely to add several years to the development timeline as compared to traditional mammalian cell culture technology. This timeline penalty can be offset by undertaking phase I and II trials with antibodies from one source, such as CHO or hybridoma, and then switching to antibodies produced in transgenic animals for phase III studies and commercialization. Such a strategy may be attractive where there is an anticipated need for very large quantities of antibody, e.g., large potential patient populations, or for chronic indications. A bridging clinical study to demonstrate equivalence of the antibodies from the different sources will likely be required. Recent advances in whole animal cloning (130), including the production of a human protein (131), also have the potential to partially offset this timeline penalty.

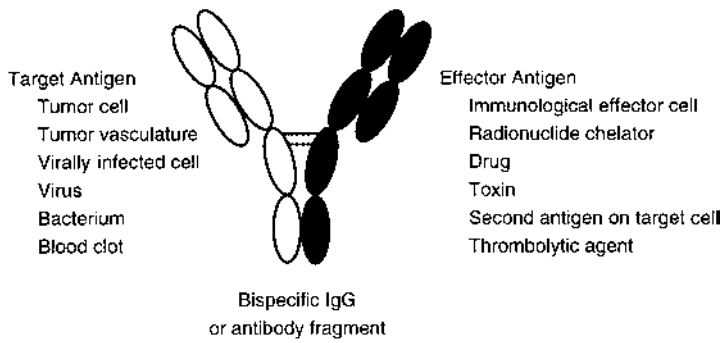
D. Transgenic Plants

Immunoglobulins have also been prepared in transgenic plants such as tobacco and corn (reviewed in Ref. 132). Indeed the first such ''plantibody'' to enter clinical trials was a secretory IgA molecule produced in transgenic tobacco directed to an antigen from *Streptococcus mutans* for the treatment of dental caries (133). The glycosylation of an antibody expressed in a plant will be substantially different from that resulting from production in mammalian cell culture. The

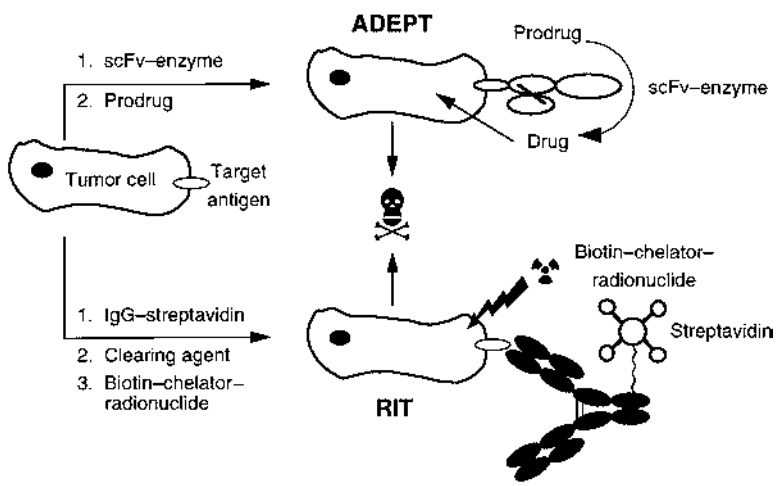
A Armed Antibodies



B Bispecific Antibodies



C Pretargeting Technologies



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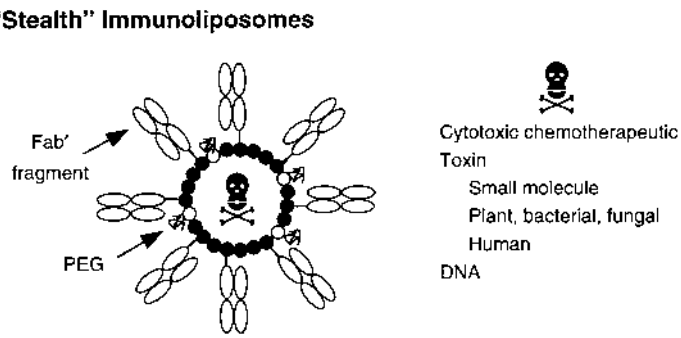

Figure 5 Technologies for enhancing the efficacy of antibody therapeutics.

impact of such glycosylation differences upon pharmacokinetics, secondary immune functions, and immunogenicity remains to be determined. This potential problem can be circumvented by the genetic removal of glycosylation site, although such aglycosylated antibodies are unlikely to support secondary immune functions. Indeed, such a genetically aglycosylated IgG produced in transgenic corn has already been tested in clinical trials and found to have similar pharmacokinetics to its parent antibody (J. Reno, personal communication). The generation and propagation of transgenic plants will likely result in longer development timelines than traditional mammalian cell culture production of antibodies.

VII. Technologies for Enhancing Antibody Therapeutics

The five marketed anticancer antibodies, Panorex, Rituxan, Herceptin, Mylotarg, and Compath, demonstrate the tremendous progress that has been made in recent years in using antibodies to treat cancer, while underscoring the need to increase the response rates and durations. Many technologies are being developed to enhance the potency of antibody therapeutics, primarily for oncology indications, including direct arming (1,3,134,135), bispecific antibodies (136), pretargeting (137–140), and immunoliposomes (141). Thirteen of 46 antibody therapeutics in advanced clinical trials in the United State utilize some enhancement technology (Table 2).

A. Direct Antibody Arming

Antibodies and their fragments have been armed in numerous ways, including with radionuclides (6,122,142,143), protein and small molecule toxins (6,144), as well as with biological response modifiers such as cytokines (145) (Fig. 5A).

Arming antibodies commonly enhances their antitumor activity in xenograft models in vivo. Only limited toxicity is usually encountered since the antibody binds the human antigen in the xenograft but commonly does not cross-react with the corresponding murine antigen. In contrast, clinical testing of armed antibodies has often resulted in unacceptably high toxicity due to cross-reactivity with antigen-expressing normal tissue (6,20,21). Thus, the judicious choice of a target antigen that is absent or present at only low levels on normal tissue is likely key to the success of antibody arming strategies. Certainly the antigens most successfully targeted with armed antibodies to date, CD20 and CD33 (see below), are very restrictive in their tissue distribution.

Conventional Radioimmunotherapy

Radioconjugates have been the most extensively studied method for arming antibodies (6,122). Indeed, at least five such radioconjugates have advanced to at least phase I/II trials (Table 2). A widely perceived advantage of radioconjugates over naked antibodies is their ability to kill bystander tumor cells, including antigen-negative cells. This is important since heterogeneous antigen expression within the tumor serves as a mechanism for drug resistance as residual tumor cells lacking the tumor antigen can proliferate following antibody therapy (40–42) (Sec. III.C). Bystander cell killing by radioconjugates is possible since the β-particles emitted by commonly used radioisotopes (^{131}I, ^{90}Y, ^{186}Rh, and ^{188}Rh) are effective over many cell diameters—nearly 4 mm in the case of ^{90}Y (143). However, the ability of MAb radioconjugates to kill cells at a distance is a double-edged sword since it also contributes to toxicity. α-Emitters such as ^{212}Bi and ^{211}At are an attractive alternative to β-emitters, since α-particles are highly cytotoxic but only over a very short range ($<$100 μm) (142,143). Unfortunately, the clinical utility of these α-emitting radionuclides is currently curtailed by their scarcity, which reflects in part their short half-lives. Many commonly used β-emitters (^{131}I, ^{186}Rh, and ^{188}Rh but not ^{90}Y) also emit γ-rays, which can conveniently be used for imaging and quantification of radionuclide uptake (146,147).

At least six radioconjugates of antibodies and antibody fragments have already been approved as clinical diagnostic agents in the United State (1). On the therapeutic front, arming anti-CD20 antibodies by radioconjugation has been convincingly shown to benefit non-Hodgkin's lymphoma patients (148–151). Moreover, two anti-CD20 radioconjugates, Bexxar (^{131}I) and Levalin (^{90}Y), have successfully completed phase III clinical trials and are anticipated to be the first radioimmunoconjugates to be approved for therapy (Table 2).

Immunotoxins

For the last two decades, immunotoxins have been constructed primarily by chemical coupling or genetic fusion of plant and bacterial toxins such as ricin A

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chain and *Pseudomonas* exotoxin, respectively, to antibodies or antibody fragments (6,144). Clinical evaluation of such immunotoxins has been plagued by problems of toxicity, particularly vascular leak syndrome, and by immunogenicity that often precludes multiple dosing. These issues notwithstanding, antitumor responses have sometimes been seen (152).

This immunogenicity problem with conventional protein toxins might potentially be overcome by the recruitment of human proteins such as ribonucleases to serve as toxins. The human ribonuclease angiogenin has been fused to anti-transferrin receptor antibody fragments and shown to be selectively cytotoxic in vitro to antigen positive cells (153). The cytotoxic activity of two other human ribonucleases has been increased ~5000-fold in vitro by site-specific chemical coupling to a ligand or antibody. This enhanced cytotoxicity reflects specific targeting as well as steric blockade of the interaction with a widely expressed ribonuclease inhibitor (154).

Conventional cytotoxic chemotherapeutics have been widely coupled to antibodies but have proved largely ineffective in patients (6). Major contributory factors are the low molar toxicity of these drugs and the small proportion of injected MAb that localizes to the tumor (10). This problem of low molar toxicity of antibody-drug conjugates has been overcome by constructing conjugates with exceedingly toxic small molecules such as calicheamicin (155,156) and maytansinoids (157), which are 100- to 1000-fold more potent than conventional chemotherapeutics. A humanized anti-CD33 antibody conjugated to calicheamicin has given a remission rate of 38% in acute myeloblastic leukemia with acceptable toxicities (156) (Table 1), which led to approval of this armed antibody.

Immunocytokines

Immunocytokines are recombinant antibody-cytokine fusion proteins that result in high local concentrations of cytokines within a tumor, thereby stimulating cellular immune responses (145). Immunocytokines containing IL-2 have proved to be highly effective in eliminating established metastases in syngeneic murine tumor models apparently by the expansion of immune effector cells (145). Such encouraging preclinical findings will likely lead to clinical evaluation of the immunocytokine concept.

B. Bispecific Antibodies

As their name suggests, bispecific antibodies bind to two different epitopes, usually on different antigens (Fig. 5B). Most current applications of bispecific antibodies target tumor cells with cytotoxic machinery including immune effector cells, radionuclides, drugs, and toxins. However, bispecific antibodies are also being used to target viral and bacterial pathogens as well as to deliver thrombolytic agents to blood clots (136).

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For many years clinical development of bispecific antibodies has been limited by the difficulty of producing these complex molecules in sufficient quantity and quality for clinical trials. The solution to these production difficulties now exists with the advent of efficient methods for preparing bispecific antibody fragments including diabodies and miniantibodies (Fig. 4) (reviewed in Ref. 96) and bispecific human IgG (Fig. 5B) (158). Small-scale clinical trials with bispecific antibodies have met with only very modest success (136). It remains to be seen if recent progress in understanding the antitumor effects of bispecific antibodies combined with improved technologies for preparing these molecules can be translated into effective treatments.

C. Pretargeting Technologies

Pretargeting technologies involve targeting of an antibody to a tumor, clearance of excess circulating antibody, and delivery of the cytotoxic agent to be selectively activated or captured at the tumor site (Fig. 5C). The commonly used cytotoxic agents are prodrugs and radionuclides. Pretargeting radionuclides and prodrugs hold the promise of greatly reduced systemic toxicity as compared to conventional RIT and cytotoxic chemotherapy, respectively.

Enzyme-Activated Prodrugs

Antibody-dependent enzyme-mediated prodrug therapy (ADEPT) is a two- or sometimes three-step approach in which an antibody-enzyme fusion protein or conjugate is infused and allowed to localize to the tumor target (reviewed in Refs. 6,137,138,140). An inactive prodrug is administered once the fusion protein has been cleared from the circulation. The prodrug is activated enzymatically within the tumor by the localized fusion protein. A major advantage of ADEPT over naked antibodies is amplification of the cytotoxic effect, including activity against neighboring tumor cells. This bystander effect is anticipated to diminish the risk of tumor cells escaping therapy by loss of target antigen expression. In addition, the cytotoxic effects of the drug beyond the tumor target should be diminished, thereby reducing toxicity as compared to systemic administration of cytotoxic chemotherapy.

ADEPT has proven to be a very effective antitumor strategy in murine xenograft models but is exceedingly difficult to translate into the clinic (6, 137,138,140). A major obstacle to ADEPT is the immunogenicity of both the bacterial enzymes that are commonly used for prodrug activation and the rodent MAb used in early clinical trials (159). Human enzymes in conjunction with humanized or human MAb should help overcome this immunogenicity problem. Encouragingly, ADEPT using a humanized antibody-human β-glucuronidase fusion protein was efficacious in mice (160). However, many potential problems

remain for ADEPT since the use of human enzymes poses risks of unwanted activation of prodrug by endogenous enzymes and interference from endogenous substrates or inhibitors. Human carboxypeptidase A1 has been cleverly engineered so that it will activate a prodrug that is not a substrate for the wild-type enzyme (161), but unfortunately it was not effective in vivo (162). Much additional progress is needed if ADEPT is to become an important treatment option for cancer patients.

Pretargeted Radioimmunotherapy

Several two-step and three-step schemes have been developed for pretargeted RIT, most of which utilize the very high-affinity interaction between streptavidin and biotin (reviewed in Refs. 139,163). Schemes using bispecific antibodies and antibody-DNA conjugates have also been used. In one version of pretargeted RIT, an antibody-streptavidin conjugate or fusion protein is first allowed to localize to a tumor target (Fig. 5C). Remaining circulating conjugate is then removed by the addition of clearing agent. Finally, a radionuclide such as ^{90}Y is delivered using a biotinylated chelator. The chelator is either captured by the antibody-streptavidin in the tumor or cleared rapidly because of its small size. A major advantage of pretargeted over conventional RIT is that much higher tumor:nontumor ratios can be achieved, thereby reducing whole body exposure to radioactivity with its attendant toxicities. Successful preclinical studies have led to small-scale clinical trials with three-step pretargeted RIT (164,165). The immunogenicity of streptavidin is a major obstacle to pretargeted RIT, which will likely preclude multiple cycles of treatment, unless it can be overcome, e.g., by PEGylation (166). Another limitation of pretargeted RIT is the large amounts of radionuclide that must be administered, since only a very small fraction is captured at the tumor target site.

D. Immunoliposomes

Liposomes are lipid bilayers that have self-assembled into small (diameter $\leq$ 100 nm) colloidal particles, thereby encapsulating some of the surrounding medium. The therapeutic potential of liposomes for the delivery of drugs, toxins, and DNA has long been appreciated but has proved difficult to translate into clinical practice. Renewed interest in liposomes has followed the advent of sterically stabilized (''stealth'') liposomes that are long-lived in vivo and by improved methods for loading and retaining drugs (167). Such ''stealth'' liposomes have been selectively targeted to tumor cells in vitro using engineered antibody fragments (168–170). Encouragingly, anti-*HER2/neu* immunoliposomes loaded with doxorubicin show greater antitumor activity than free drug or drug loaded in nontargeted liposomes in several tumor xenograft models (141,170). Moreover, the systemic

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toxicity of the immunoliposome-targeted doxorubicin was much less than that of free doxorubicin. Despite such progress with immunoliposomes, several underlying difficulties remain, including their complexity and inefficient extravasation due to their large size (141).

VIII. Trends with Antibody Therapeutics

Antibodies are an important new class of drugs whose impact will doubtless increase as some additional antibodies in advanced clinical trials (Table 2) progress through to regulatory approval. Historically, a high proportion of antibodies in the clinic has been targeted to cancer and has met with very modest success. The tide is finally turning with the approvals of four antibody therapeutics for oncological indications in the United States (Table 1) and a fifth, Panorex, in Europe.

Despite these successes, great need exists for enhancing the efficacy of antibodies, particularly for tumor immunotherapy. This need has fueled the development of new technologies to enhance antibody potency (Sec. VII) (Fig. 5). The concept of antibody arming is finally coming of age with the approval of the anti-CD33-calicheamicin conjugate, Mylotarg (Table 1), and with two anti-CD20 radioconjugates, Bexxar and Zevalin, in late stages of regulatory review (Table 2). The greatest challenge to antibody arming is the identification of new antigens that are located on the target but few, or ideally no, normal tissues in order to minimize toxicity. Ideally, this effort will capitalize upon the rapid identification of new human proteins from the human genome project.

An exceedingly important trend is the successful use of antibodies as therapeutics for indications outside of oncology. Indeed, 6 of the 10 antibodies approved in the United States are for diverse nononcological indications and are based upon extensive knowledge of disease etiology. Future approvals are expected to increase the usage of antibodies in existing therapeutic areas and broaden the scope of antibodies to new areas. For example, the humanized anti-IgE antibody E25 (171), now known as Xolair, has proved efficacious in phase III trials in seasonal allergic rhinitis (172) and also in phase II (173) and phase III trials (174–177) for allergic asthma. Xolair has been submitted for regulatory approval on the basis of these promising clinical data.

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References

1. King DJ, Adair JR. Recombinant antibodies for the diagnosis and therapy of human disease. Curr Opin Drug Discov Develop 1999; 2:110–117.
2. Holliger P, Hoogenboom H. Antibodies come back from the brink. Nat Biotechnol 1998; 16:1015–1016.
3. Glennie MJ, Johnson PW. Clinical trials of antibody therapy. Immunol Today 2000; 21:403–410.
4. Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C, eds. In: Sequences of Proteins of Immunological Interest. 5th ed. Vol. 1. Bethesda, MD: National Institutes of Health, 1991:xlv.
5. Roitt I, Brostoff J, Male E. Immunology. 5th ed. London: Mosby; 1998.
6. Farah RA, Clinchy B, Herrera L, Vitetta ES. The development of monoclonal antibodies for the therapy of cancer. Crit Rev Eukary Gene Express 1998; 8:321–356.
7. Ghetie V, Popov S, Borvak J, Radu C, Matesoi D, Medesan C, Ober RJ, Ward ES. Increasing the serum persistence of an IgG fragment by random mutagenesis. Nat Biotechnol 1997; 15:637–640.
8. Medesan C, Matesoi D, Radu C, Ghetie V, Ward ES. Delineation of the amino acid residues involved in transcytosis and catabolism of mouse IgG1. J Immunol 1997; 158:2211–2217.
9. Medesan C, Cianga P, Mummert M, Stanescu D, Ghetie V, Ward ES. Comparative studies of rat IgG to further delineate the Fc:FcRn interaction site. Eur J Immunol 1998; 28:2092–2100.
10. Sedalacek H-H, Seemann G, Hoffmann D, Czech J, Lorenz P, Kolar C, Bosslet K. Antibodies as Carriers of Cytotoxicity. Contributions to Oncology. Vol. 43. Munich, Germany: Karger, 1992.
11. Miller RA, Oseroff AR, Stratte PT, Levy R. Monoclonal antibody therapeutic trials in seven patients with T-cell lymphoma. Blood 1983; 62:988–995.
12. Schroff RW, Foon KA, Beatty SM, Oldham RK, Morgan Jr AC. Human antimurine immunoglobulin responses in patients receiving monoclonal antibody therapy. Cancer Res 1985; 45:879–885.
13. Khazaeli MB, Conry RM, LoBuglio AF. Human immune response to monoclonal antibodies. J Immunother 1994; 15:42–52.
14. Bajorin DF, Chapman PB, Wong GY, Cody BV, Cordon-Cardo C, Dantes L, Templeton MA, Scheinberg D, Oettgen HF, Houghton AN. Treatment with high dose mouse monoclonal (anti-GD3) antibody R24 in patients with metastatic melanoma. Melanoma Res 1992; 2:355–362.
15. Frodin JE, Faxas ME, Hagstrom B, Lefvert AK, Masucci G, Nilsson B, Steinitz M, Unger P, Mellstedt H. Induction of anti-idiotypic (ab2) and anti-anti-idiotypic (ab3) antibodies in patients treated with the mouse monoclonal antibody 17-1A (ab1). Relation to the clinical outcome—an important antitumoral effector function? Hybridoma 1991; 10:421–431.
16. Mittelman A, Chen ZJ, Yang H, Wong GY, Ferone S. Human high molecular weight-melanoma associated antigen (HMW-MAA) mimicry by mouse anti-idiotypic monoclonal antibody MK2-23: induction of humoral anti-HMW-MAA immu-

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nity and prolongation of survival in patients with stage IV melanoma. Proc Natl Acad Sci USA 1992; 89:466–470.
17. Schultes BC, Yang R, Agopsowicz K, Kuzma M, Dharampaul S, Baum RP, Noujaim AA, Madiyalakan R. Anti-idiotype induction therapy for ovarian cancer: immune responses in patients injected with OvaRex™-MAb-B43.13. Proc Annu Meet Am Soc Clin Oncol 1999; 18:A1399.
18. Schultes BC, Baum RP, Niesen A, Noujaim AA, Madiyalakan R. Anti-idiotype induction therapy: anti-CA125 antibodies (Ab3) mediated tumor killing in patients treated with Ovarex mAb B43.13 (Ab1). Cancer Immunol Immunother 1998; 46: 201–212.
19. Dillman RO. Antibody therapy. In: Oldham RK, ed. Principles of Cancer Biotherapy. 3rd ed. Dordrecht: Kluwer Academic Publishers, 1998:284–317.
20. Gould BJ, Borowitz MJ, Groves ES, Carter PW, Anthony D, Weiner LM, Frankel AE. Phase I study of an anti–breast cancer immunotoxin by continuous infusion: report of a targeted toxic effect not predicted by animal studies. J Natl Cancer Inst 1989; 81:775–781.
21. Pai LH, Bookman MA, Ozols RF, Young RC, Smith JW, Longo DL, Gould B, Frankel A, McClay EF, Howell S, Reed E, Willingham MC, FitzGerald DJ, Pastan I. Clinical evaluation of intraperitoneal *Pseudomonas* exotoxin immunoconjugate OVB3-PE in patients with ovarian cancer. J Clin Oncol 1991; 9:2095–2103.
22. Maloney DG, Press OW. Newer treatments for non-Hodgkin's lymphoma: monoclonal antibodies. Oncology 1998; 12(suppl):63–76.
23. Jain RK. Physiological barriers to delivery of monoclonal antibodies and other macromolecules in tumors. Cancer Res 1990; 50:814s–819s.
24. Dvorak HF, Nagy JA, Dvorak AM. Structure of solid tumors and their vasculature: implications for therapy with monoclonal antibodies. Cancer Cells 1991; 3:77–85.
25. Baxter LT, Yuan F, Jain RK. Pharmacokinetic analysis of the perivascular distribution of bifunctional antibodies and haptens: comparison with experimental data. Cancer Res 1992; 52:5838–5844.
26. Adams GP. Improving the tumor specificity and retention of antibody-based molecules. In vivo 1998; 12:11–22.
27. Buchegger F, Halpern SE, Sutherland RM, Schreyer M, Mach J-P. In vitro and in vivo tumor models for studies of distribution of radiolabelled monoclonal antibodies and fragments. Nuklearmedizin 1986; 25:207–209.
28. Juweid M, Neumann R, Paik C, Perez-Bacete MJ, Sato J, van Osdol W, Weinstein JN. Micropharmacology of monoclonal antibodies in solid tumors: direct experimental evidence for a binding site barrier. Cancer Res 1992; 52:5144–5153.
29. Fujimori K, Covell DG, Fletcher JE, Weinstein JN. Modeling analysis of the global and microscopic distribution of immunoglobulin G, $F(ab')_2$, and Fab in tumors. Cancer Res 1989; 49:5656–5663.
30. Oldham RK, Foon KA, Morgan AC, Woodhouse CS, Schroff RW, Abrams PG, Fer M, Schoenberger CS, Farrell M, Kimball E, Sherwin SA. Monoclonal antibody therapy of malignant melanoma: in vivo localization in cutaneous metastases after intravenous administration. J Clin Oncol 1984; 2:1235–1244.
31. Glassy MC, Dillman RO. Molecular biotherapy with human monoclonal antibodies. Mol Biother 1988; 1:7–13.

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32. Dillman RO. Antibodies as cytotoxic therapy. J Clin Oncol 1994; 12:1497–1515.
33. Langmuir VK, Mendonca HL, Woo DV. Comparisons between two monoclonal antibodies that bind to the same antigen but have differing affinities: uptake kinetics and ^{125}I-antibody therapy efficacy in multicell spheroids. Cancer Res 1992; 52: 4728–4734.
34. Colcher DFM, Roselli M, Muraro R, Simpson-Milenic D, Schlom J. Radioimmunolocalization of human carcinoma xenografts with B72.3 second generation monoclonal antibodies. Cancer Res 1988; 48:4597–4603.
35. Adams GP, Schier R, Marshall K, Wolf EJ, McCall AM, Marks JD, Weiner LM. Increased affinity leads to improved selective tumor delivery of single-chain Fv antibodies. Cancer Res 1998; 58:485–490.
36. Kuhn JA, Wong JYC, Beatty BG, Esteban JM, Williams LE, Beatty JD. Interferon enhancement of carcinoembryonic antigen expression in human colon carcinoma xenografts. J Immunother 1992; 11:257–266.
37. Burrows FJ, Thorpe PE. Eradication of large solid tumors in mice with an immunotoxin directed against tumor vasculature. Proc Natl Acad Sci USA 1993; 90:8996–9000.
38. Huang X, Molema G, King S, Watkins L, Edgington TS, Thorpe PE. Tumor infarction in mice by antibody-directed targeting of tissue factor to tumor vasculature. Science 1997; 275:547–550.
39. Nilsson F, Kosmehl H, Zardi L, Neri D. Targeted delivery of tissue factor to the ED-B domain of fibronectin, a marker of angiogenesis, mediates the infarction of solid tumors in mice. Cancer Res 2001; 61:711–716.
40. Meeker T, Lowder J, Cleary ML, Stewart S, Warnke R, Sklar J, Levy R. Emergence of idiotype variants during treatment of B-cell lymphoma with anti-idiotype antibodies. N Engl J Med 1985; 312:1658–1665.
41. Brett SJ, Baxter G, Cooper H, Rowan W, Regan T, Tite J, Rapson N. Emergence of CD52-, glycosylphosphatidylinositol-anchor-deficient lymphocytes in rheumatoid arthritis patients following Campath-1H treatment. Internat Immunol 1996; 8:325–334.
42. Davis TA, Czerwinski DK, Levy R. Therapy of B-cell lymphoma with anti-CD20 antibodies can result in the loss of CD20 antigen expression. Clin Cancer Res 1999; 5:611–615.
43. Garnick RL, Ross MJ, Baffi RA. Characterization of proteins from recombinant DNA manufacture. In: Chiu YH, Gueriguian JL, eds. Drug Biotechnology Regulation: Scientific Basis and Practices. New York: Marcel Dekker, 1991:263–313.
44. Geigert J. Overview of the stability and handling of recombinant protein drugs. J Parenter Sci Technol 1989; 43:220–224.
45. Smith SL. Ten years of orthoclone OKT3 (muromonab-CD3): a review. J Transplant Coord 1996; 6:109–121.
46. Byrd JC, Waselenko JK, Maneatis TJ, Murphy T, Ward FT, Monahan BP, Sipe MA, Donegan S, White CA. Rituximab therapy in hematologic malignancy patients with circulating blood tumor cells: association with increased infusion-related side effects and rapid blood tumor clearance. J Clin Oncol 1999; 17:791–795.
47. Anderson JA, Adkinson NF. Allergic reactions to drugs and biologic agents. J Am Med Assoc 1987; 258:2891–2899.

MARCEL DEKKER, INC.
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48. van Arsdel PP. Classification and risk factors for drug allergy. Immunol Allergy Clin North Am 1991; 11:475–492.
49. Dillman RO. Human antimouse and antiglobulin responses to monoclonal antibodies. Antibody Immunoconjug Radiopharm 1990; 3:1–15.
50. Vaughan TJ, Osbourn JK, Tempest PR. Human antibodies by design. Nat Biotechnol 1998; 16:535–539.
51. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975; 256:495–497.
52. Herlyn M, Steplewski Z, Herlyn D, Koprowski H. Colorectal carcinoma-specific antigen: detection by means of monoclonal antibodies. Proc Natl Acad Sci USA 1979; 76:1438–1442.
53. Riethmüller G, Schneider-Gädicke E, Schlimok G, Schmiegel W, Raab R, Höffken K, Gruber R, Pichlmaier H, Hirche H, Pichlmayr R. Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. German Cancer Aid 17-1A Study Group. Lancet 1994; 843:1177–1183.
54. Riethmüller G, Holz E, Schlimok G, Schmiegel W, Raab R, Höffken K, Gruber R, Funke I, Pichlmaier H, Hirche H, Buggisch P, Witte J, Pichlmayr R. Monoclonal antibody therapy for resected Dukes' C colorectal cancer: seven-year outcome of a multicenter randomized trial. J Clin Oncol 1998; 16:1788–1794.
55. Mellstedt H, Frodin JE, Masucci G, Ragnhammar P, Fagerberg J, Hjelm AL, Shetye J, Wersall P, Österborg A. The therapeutic use of monoclonal antibodies in colorectal carcinoma. Semin Oncol 1991; 18:462–477.
56. Boulianne GL, Hozumi N, Shulman MJ. Production of functional chimaeric mouse/human antibody. Nature 1984; 312:643–646.
57. Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. Proc Natl Acad Sci USA 1984; 81:6851–6855.
58. Brüggemann M, Williams GT, Bindon CI, Clark MR, Walker MR, Jefferis R, Waldmann H, Neuberger MS. Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. J Exp Med 1987; 166: 1351–1361.
59. LoBuglio AF, Wheeler RH, Trang J, Haynes A, Rogers K, Harvey EB, Sun L, Ghrayeb J, Khazaeli MB. Mouse/human chimeric monoclonal antibody in man: kinetics and immune response. Proc Natl Acad Sci USA 1989; 86:4220–4224.
60. Goodman GE, Hellstrom I, Yelton DE, Murray JL, O'Hara S, Meaker E, Zeigler L, Palazollo P, Nicaise C, Usakewicz J, Hellstrom KE. Phase I trial of chimeric (human-mouse) monoclonal antibody L6 in patients with non-small-cell lung, colon, and breast cancer. Cancer Immunol Immunother 1993; 36:267–273.
61. Maloney DG, Grillo-López AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, Janakiraman N, Foon KA, Liles TM, Dallaire BK, Wey K, Royston I, Davis T, Levy R. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood 1997; 90:2188–2195.
62. McLaughlin P, Grillo-López AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK. Rituximab chimeric anti-CD20 monoclonal antibody

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270 Madison Avenue, New York, New York 10016

therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998; 16:2825–2833.
63. EPIC Investigators. Use of a monoclonal antibody directed against the platelet glycoprotein II_b/III_a receptor in high risk coronary angioplasty. N Engl J Med 1994; 330:956–961.
64. Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 1986; 321:522–525.
65. Riechmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. Nature 1988; 332:323–327.
66. Verhoeyen M, Milstein C, Winter G. Reshaping human antibodies: grafting an antilysozyme activity. Science 1988; 239:1534–1536.
67. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L. Phase II study of weekly intravenous recombinant humanized anti-$p185^{HER2}$ monoclonal antibody in patients with HER2/*neu*-overexpressing metastatic breast cancer. J Clin Oncol 1996; 14:737–744.
68. Caron PC, Jurcic JG, Scott AM, Finn RD, Divgi CR, Graham MC, Jureidini IM, Sgouros G, Tyson D, Old LJ, et al. A phase 1B trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: specific targeting without immunogenicity. Blood 1994; 83:1760–1768.
69. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, Baly D, Baughman SA, Twaddell T, Glaspy JA, Slamon DJ. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-$p185^{HER2/neu}$ monoclonal antibody plus cisplatin in patients with HER2/*neu*-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J Clin Oncol 1998; 16:2659–2671.
70. Sharkey RM, Juweid M, Shevitz J, Behr T, Dunn R, Swayne LC, Wong GY, Blumenthal RD, Griffiths GL, Siegel JA, Leung S-o, Hansen HJ, Goldenberg DM. Evaluation of a complementarity-determining region-grafted (humanized) anti-carcinoembryonic antigen monoclonal antibody in preclinical and clinical studies. Cancer Res 1995; 55:5935s–5945s.
71. Isaacs JD, Watts RA, Hazleman BL, Hale G, Keogan MT, Cobbold SP, Waldmann H. Humanised monoclonal antibody therapy for rheumatoid arthritis. Lancet 1992; 340:748–752.
72. Richards J, Auger J, Peace D, Gale D, Michel J, Koons A, Haverty T, Zivin R, Jolliffe L, Bluestone JA. Phase I evaluation of humanized OKT3: toxicity and immunomodulatory effects of hOKT3 γ4. Cancer Res 1999; 59:2096–2101.
73. Vincenti F, Lantz M, Birnbaum J, Garovoy M, Mould D, Hakimi J, Nieforth K, Light S. A phase I trial of humanized anti-interleukin 2 receptor antibody in renal transplantation. Transplant 1997; 63:33–38.
74. Nashan B, Light S, Hardie IR, Lin A, Johnson JR. Reduction of acute renal allograft rejection by daclizumab. Transplant 1999; 67:110–115.
75. Meanwell NA, Krystal M. Respiratory syncytial virus: recent progress towards the discovery of effective prophylactic and therapeutic agents. Drug Disc Today 2000; 5:241–252.

MARCEL DEKKER, INC.
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76. Hale G, Dyer MJ, Clark MR, Phillips JM, Marcus R, Riechmann L, Winter G, Waldmann H. Remission induction in non-Hodgkin lymphoma with reshaped human monoclonal antibody CAMPATH-1H. Lancet 1988; 2:1394–1399.
77. Carter P, Presta L, Gorman CM, Ridgway JB, Henner D, Wong WL, Rowland AM, Kotts C, Carver ME, Shepard HM. Humanization of an anti-p185^{HER2} antibody for human cancer therapy. Proc Natl Acad Sci USA 1992; 89:4285–4289.
78. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344:783–792.
79. Marks JD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD, Winter G. By-passing immunization. Human antibodies from V-gene libraries displayed on phage. J Mol Biol 1991; 222:581–597.
80. de Haard HJ, van Neer N, Reurs A, Hufton SE, Roovers RC, Henderikx P, de Bruine AP, Arends JW, Hoogenboom HR. A large non-immunized human Fab fragment phage library that permits rapid isolation and kinetic analysis of high affinity antibodies. J Biol Chem 1999; 274:18218–18230.
81. Griffiths AD, Williams SC, Hartley O, Tomlinson IM, Waterhouse P, Crosby WL, Kontermann RE, Jones PT, Low NM, Allison TJ, Prospero TD, Hoogenboom HR, Nissim A, Cox JPL, Harrison JL, Zaccolo M, Gherardi E, Winter G. Isolation of high affinity human antibodies directly from large synthetic repertoires. EMBO J 1994; 13:3245–3260.
82. Knappik A, Ge L, Honegger A, Pack P, Fischer M, Wellnhofer G, Hoess A, Wolle J, Plückthun A, Virnekas B. Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides. J Mol Biol 2000; 296:57–86.
83. Sheets MD, Amersdorfer P, Finnern R, Sargent P, Lindquist E, Schier R, Hemingsen G, Wong C, Gerhart JC, Marks JD. Efficient construction of a large nonimmune phage antibody library: the production of high-affinity human single-chain antibodies to protein antigens. Proc Natl Acad Sci USA 1998; 95:6157–6162.
84. Sblattero D, Bradbury A. Exploiting recombination in single bacteria to make large phage antibody libraries. Nat Biotechnol 2000; 18:75–80.
85. Söderlind E, Strandberg L, Jirholt P, Kobayashi N, Alexeiva V, Aberg AM, Nilsson A, Jansson B, Ohlin M, Wingren C, Danielsson L, Carlsson R, Borrebaeck CA. Recombining germline-derived CDR sequences for creating diverse single-framework antibody libraries. Nat Biotechnol 2000; 18:852–856.
86. Yang W-P, Green K, Pinz-Sweeney S, Briones AT, Burton DR, Barbas CF, 3rd. CDR walking mutagenesis for the affinity maturation of a potent human anti-HIV-1 antibody into the picomolar range. J Mol Biol 1995; 254:392–403.
87. Schier R, McCall A, Adams GP, Marshall KW, Merritt H, Yim M, Crawford RS, Weiner LM, Marks C, Marks JD. Isolation of picomolar affinity anti-c-erbB-2 single-chain Fv by molecular evolution of the complementarity determining regions in the center of the antibody binding site. J Mol Biol 1996; 263:551–567.
88. Jermutus L, Ryabova LA, Plückthun A. Recent advances in producing and selecting functional proteins by using cell-free translation. Curr Opin Biotechnol 1998; 9: 534–548.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

89. Hanes J, Schaffitzel C, Knappik A, Plückthun A. Picomolar affinity antibodies from a fully synthetic naive library selected and evolved by ribosome display. Nat Biotechnol 2000; 18:1287–1292.
90. Brüggemann M, Taussig MJ. Production of human antibody repertoires in transgenic mice. Curr Opin Biotechnol 1997; 8:455–458.
91. Fishwild DM, O'Donnel lSL, Bengoechea T, Hudson DV, Harding F, Bernhard SL, Jones D, Kay RM, Higgins KM, Schramm SR, Lonberg N. High avidity human IgGκ monoclonal antibodies from a novel strain of minilocus transgenic mice. Nat Biotechnol 1996; 14:845–851.
92. Mendez MJ, Green LL, Corvalan JRF, Jia XC, Maynard-Currie CE, Yang X-D, Gallo ML, Louie DM, Lee DV, Erickson KL, Luna J, Roy CMN, Abderrahim H, Kirschenbaum F, Noguchi M, Smith DH, Fukushima A, Hales JF, Finer MH, Davis CG, Zsebo KM, Jakobovits A. Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice. Nat Genetics 1997; 15:146–156.
93. Tomizuka K, Shinohara T, Yoshida H, Uejima H, Ohguma A, Tanaka S, Sato K, Oshimura M, Ishida I. Double trans-chromosomic mice: maintenance of two individual human chromosome fragments containing Ig heavy and κ loci and expression of fully human antibodies. Proc Natl Acad Sci USA 2000; 97:722–727.
94. Nicholson IC, Zou X, Popov AV, Cook GP, Corps EM, Humphries S, Ayling C, Goyenechea B, Xian J, Taussig MJ, Neuberger MS, Brüggemann M. Antibody repertoires of four- and five-feature translocus mice carrying human immunoglobulin heavy chain and κ and λ light chain yeast artificial chromosomes. J Immunol 1999; 163:6898–6906.
95. Yang XD, Jia XC, Corvalan JRF, Wang P, Davis CG, Jakobovits A. Eradication of established tumors by a fully human monoclonal antibody to the epidermal growth factor receptor without concomitant chemotherapy. Cancer Res 1999; 59: 1236–1243.
96. Plückthun A, Pack P. New protein engineering approaches to multivalent and bispecific antibody fragments. Immunotechnology 1997; 3:83–105.
97. Parham P. Preparation and purification of active fragments from mouse monoclonal antibodies. In: Weir EM, ed. Cellular Immunology. 4th ed. Vol II. Edinburgh: Blackwell Scientific Press, 1983:1401–1423.
98. Plückthun A, Krebber A, Krebber C, Horn U, Knüpfer U, Wenderoth R, Nieba L, Proba K, Riesenberg D. Producing antibodies in *Escherichia coli*: from PCR to fermentation. In: Hoogenboom H, McCafferty J, Chiswell D, eds. Antibody Engineering: A Practical Approach. Oxford, UK: IRL Press, 1996:203–252.
99. Bird RE, Hardman KD, Jacobson JW, Johnson S, Kaufman BM, Lee SM, Lee T, Pope SH, Riordan GS, Whitlow M. Single-chain antigen-binding proteins. Science 1988; 242:423–426.
100. Huston JS, Levinson D, Mudgett-Hunter M, Tai MS, Novotny J, Margolies MN, Ridge RJ, Bruccoleri RE, Haber E, Crea R, Oppermann H. Protein engineering of antibody binding sites: recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in *Escherichia coli*. Proc Natl Acad Sci USA 1988; 85: 5879–5883.
101. Gruber M, Schodin BA, Wilson ER, Kranz DM. Efficient tumor cell lysis mediated

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by a bispecific single chain antibody expressed in *Escherichia coli*. J Immunol 1994; 152:5368–5374.
102. Mallender WD, Voss JEW. Construction, expression, and activity of a bivalent bispecific single-chain antibody. J Biol Chem 1994; 269:199–206.
103. Cumber A, Ward ES, Winter G, Parnell GD, Wawrzynczak EJ. Comparative stabilities in vitro and in vivo of a recombinant mouse antibody FvCys fragment and a bisFvCys conjugate. J Immunol 1992; 149:120–126.
104. Hu S, Shively L, Raubitschek A, Sherman M, Williams LE, Wong JY, Shively JE, Wu AM. Minibody: a novel engineered anti-carcinoembryonic antigen antibody fragment (single-chain Fv-C_H3) which exhibits rapid, high-level targeting of xenografts. Cancer Res 1996; 56:3055–3061.
105. Pack P, Plückthun A. Miniantibodies: use of amphipathic helices to produce functional, flexibly linked dimeric FV fragments with high avidity in *Escherichia coli*. Biochemistry 1992; 31:1579–1584.
106. Pack P, Kujau M, Schroeckh V, Knüpfer U, Wenderoth R, Riesenberg D, Plückthun A. Improved bivalent miniantibodies, with identical avidity as whole antibodies, produced by high cell density fermentation of *Escherichia coli*. Bio/Technol 1993; 11:1271–1277.
107. Pack P, Müller K, Zahn R, Plückthun A. Tetravalent miniantibodies with high avidity assembling in *Escherichia coli*. J Mol Biol 1995; 246:28–34.
108. Müller KM, Arndt KM, Plückthun A. A dimeric bispecific miniantibody combines two specificities with avidity. FEBS Lett 1998; 432:45–49.
109. Holliger P, Prospero T, Winter G. ''Diabodies'': small bivalent and bispecific antibody fragments. Proc Natl Acad Sci USA 1993; 90:6444–6448.
110. Zhu Z, Zapata G, Shalaby R, Snedecor B, Chen H, Carter P. High level secretion of a humanized bispecific diabody from *Escherichia coli*. Bio/Technol 1996; 14: 192–196.
111. Holliger P, Wing M, Pound JD, Bohlen H, Winter G. Retargeting serum immunoglobulin with bispecific diabodies. Nat Biotechnol 1997; 15:632–636.
112. Kontermann RE, Wing MG, Winter G. Complement recruitment using bispecific diabodies. Nat Biotechnol 1997; 15:629–631.
113. Begent RH, Verhaar MJ, Chester KA, Casey JL, Green AJ, Napier MP, Hope-Stone LD, Cushen N, Keep PA, Johnson CJ, Hawkins RE, Hilson AJ, Robson L. Clinical evidence of efficient tumor targeting based on single-chain Fv antibody selected from a combinatorial library. Nat Med 1996; 2:979–984.
114. Rhee P, Morris J, Durham R, Hauser C, Cipolle M, Wilson R, Luchette F, McSwain N, Miller R. Recombinant humanized monoclonal antibody against CD18 (rhuMAb CD18) in traumatic hemorrhagic shock: results of a phase II clinical trial. J Trauma Inj Infect Crit Care 2000; 49:611–619.
115. Lewis GD, Figari I, Fendly B, Wong WL, Carter P, Gorman C, Shepard HM. Differential responses of human tumor cell lines to anti-$p185^{HER2}$ monoclonal antibodies. Cancer Immunol Immunother 1993; 37:255–263.
116. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat Med 2000; 6:443–446.
117. Zapata G, Ridgway JB, Mordenti J, Osaka G, Wong WL, Bennett GL, Carter P.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Engineering linear $F(ab')_2$ fragments for efficient production in *Escherichia coli* and enhanced antiproliferative activity. Protein Engin 1995; 8:1057–1062.
118. Yokota T, Milenic DE, Whitlow M, Schlom J. Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. Cancer Res 1992; 52: 3402–3408.
119. Colcher D, Bird R, Roselli M, Hardman KD, Johnson S, Pope S, Dodd SW, Pantoliano MW, Milenic DE, Schlom J. In vivo tumor targeting of a recombinant single-chain antigen-binding protein. J Natl Cancer Inst 1990; 82:1191–1197.
120. Milenic DE, Yokota T, Filpula DR, Finkelman MA, Dodd SW, Wood JF, Whitlow M, Snoy P, Schlom J. Construction, binding properties, metabolism, and tumor targeting of a single-chain Fv derived from the pancarcinoma monoclonal antibody CC49. Cancer Res 1991; 51:6363–6371.
121. Savage P, Rowlinson-Busza G, Verhoeyen M, Spooner RA, So A, Windust J, Davis PJ, Epenetos AA. Construction, characterisation and kinetics of a single chain antibody recognising the tumour associated antigen placental alkaline phosphatase. Br J Cancer 1993; 68:738–742.
122. Wilder RB, DeNardo GL, DeNardo SJ. Radioimmunotherapy: recent results and future directions. J Clin Oncol 1996; 14:1383–1400.
123. Adair JR. Antibody engineering and expression. In: Mountain A, Ney U, Schomburg D, eds. Recombinant Proteins, Monoclonal Antibodies and Therapeutic Genes. Vol. 5a. Biotechnology. Weinheim, Germany: Wiley-VCH, 1999:219–244.
124. Chadd HE, Chamow SM. Therapeutic antibody expression technology. Curr Opin Biotechnol 2001; 12:188–194.
125. Garber K. Biotech industry faces new bottleneck. Nat Biotechnol 2001; 19:184–185.
126. Carter P, Kelley RF, Rodrigues ML, Snedecor B, Covarrubias M, Velligan MD, Wong WL, Rowland AM, Kotts CE, Carver ME, Yang M, Bourell JH, Shepard HM, Henner D. High level *Escherichia coli* expression and production of a bivalent humanized antibody fragment. Bio/Technol 1992; 10:163–167.
127. Horn U, Strittmatter W, Krebber A, Knüpfer U, Kujau M, Wenderoth R, Müller K, Matzku S, Plückthun A, Riesenberg D. High volumetric yields of functional dimeric miniantibodies in *Escherichia coli*, using an optimized expression vector and high-cell-density fermentation under non-limited growth conditions. Appl Microbiol Biotechnol 1996; 46:524–532.
128. Chapman AP, Antoniw P, Spitali M, West S, Stephens S, King DJ. Therapeutic antibody fragments with prolonged in vivo half-lives. Nat Biotechnol 1999; 17: 780–783.
129. Ward RL, Hawkins NJ, Smith GM. Unconjugated antibodies for cancer therapy: lessons from the clinic. Cancer Treat Rev 1997; 23:305–319.
130. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. Nature 1997; 385:810–813.
131. Schnieke AE, Kind AJ, Ritchie WA, Mycock K, Scott AR, Ritchie M, Wilmut I, Colman A, Campbell KH. Human factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. Science 1997; 278:2130–2133.
132. Franken E, Teuschel U, Hain R. Recombinant proteins from transgenic plants. Curr Opin Biotechnol 1997; 8:411–416.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

133. Ma JK-C, Hikmat BY, Wycoff K, Vine ND, Chargelegue D, Yu L, Hein MB, Lehner T. Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat Med 1998; 4:601–606.
134. Carter P, Merchant AM. Engineering antibodies for imaging and therapy. Curr Opin Biotechnol 1997; 8:449–454.
135. Dall'Acqua W, Carter P. Antibody engineering. Curr Opin Struct Biol 1998; 8: 443–450.
136. van de Winkel JG, Bast B, de Gast GC. Immunotherapeutic potential of bispecific antibodies. Immunol Today 1997; 18:562–564.
137. Niculescu-Duvaz I, Springer CJ. Antibody-directed enzyme prodrug therapy (ADEPT): a review. Adv Drug Deliver Rev 1997; 26:151–172.
138. Niculescu-Duvaz I, Friedlos F, Niculescu-Duvaz D, Davies L, Springer CJ. Prodrugs for antibody- and gene-directed enzyme prodrug therapies (ADEPT and GDEPT). Anti-Cancer Drug Design 1999; 14:517–538.
139. Stoldt HS, Aftab F, Chinol M, Paganelli G, Luca F, Testori A, Geraghty JG. Pretargeting strategies for radio-immunoguided tumour localisation and therapy. Eur J Cancer 1997; 33:186–192.
140. Syrigos KN, Epenetos AA. Antibody directed enzyme prodrug therapy (ADEPT): a review of the experimental and clinical considerations. Anticancer Res 1999; 19: 605–613.
141. Park JW, Hong K, Kirpotin DB, Papahadjopoulos D, Benz CC. Immunoliposomes for cancer treatment. Adv Pharmacol 1997; 40:399–435.
142. McDevitt MR, Sgouros G, Finn RD, Humm JL, Jurcic JG, Larson SM, Scheinberg DA. Radioimmunotherapy with α-emitting nuclides. Eur J Nuc Med 1998; 25: 1341–1351.
143. Zalutsky MR, Bigner DD. Radioimmunotherapy with α-particle emitting radioimmunoconjugates. Acta Oncol 1996; 35:373–379.
144. Pastan I. Targeted therapy of cancer with recombinant immunotoxins. Biochim Biophys Acta 1997; 333:C1–6.
145. Lode HN, Xiang R, Becker JC, Gillies SD, Reisfeld RA. Immunocytokines: a promising approach to cancer immunotherapy. Pharmacol Ther 1998; 80:277–292.
146. Goldenberg DM. Advances in cancer imaging and therapy with radiolabelled antibodies. In: Baum RP, et al., eds. Clinical Uses of Antibodies. Dordrecht, The Netherlands: Kluwer Academic Press, 1991:1–13.
147. Jurcic JG, Scheinberg DA. Recent developments in the radioimmunotherapy of cancer. Curr Opin Immunol 1994; 6:715–721.
148. Kaminski MS, Zasadny KR, Francis IR, Milik AW, Ross CW, Moon SD, Crawford SM, Burgess JM, Petry NA, Butchko GM. Radioimmunotherapy of B-cell lymphoma with [^{131}I]anti-B1 (anti-CD20) antibody. N Engl J Med 1993; 329:459–465.
149. Kaminski MS, Zasadny KR, Francis IR, Fenner MC, Ross CW, Milik AW, Estes J, Tuck M, Regan D, Fisher S, Glenn SD, Wahl RL. Iodine-131-anti-B1 radioimmunotherapy for B-cell lymphoma. J Clin Oncol 1996; 14:1974–1981.
150. Press OW, Eary JF, Appelbaum FR, Martin PJ, Badger CC, Nelp WB, Glenn S, Butchko G, Fisher D, Porter B. Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. N Engl J Med 1993; 329:1219–1224.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

151. Press OW, Eary JF, Appelbaum FR, Martin PJ, Nelp WB, Glenn S, Fisher DR, Porter B, Matthews DC, Gooley T. Phase II trial of ^{131}I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. Lancet 1995; 346:336–340.
152. Pai LH, Wittes R, Setser A, Willingham MC, Pastan I. Treatment of advanced solid tumors with immunotoxin LMB-1: an antibody linked to *Pseudomonas* exotoxin. Nat Med 1996; 2:350–353.
153. Rybak SM, Hoogenboom HR, Meade HM, Raus JC, Schwartz D, Youle RJ. Humanization of immunotoxins. Proc Natl Acad Sci USA 1992; 89:3165–3169.
154. Suzuki M, Saxena SK, Boix E, Prill RJ, Vasandani VM, Ladner JE, Sung C, Youle RJ. Engineering receptor-mediated cytotoxicity into human ribonucleases by steric blockade of inhibitor interaction. Nat Biotechnol 1999; 17:265–270.
155. Hinman LM, Hamann PR, Wallace R, Menendez AT, Durr FE, Upeslacis J. Preparation and characterization of monoclonal antibody conjugates of the calicheamicins: a novel and potent family of antitumor antibiotics. Cancer Res 1993; 53:3336–3342.
156. Sievers EL, Appelbaum FR, Spielberger RT, Forman SJ, Flowers D, Smith FO, Shannon-Dorcy K, Berger MS, Bernstein ID. Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: a phase I study of an anti-CD33 calicheamicin immunoconjugate. Blood 1999; 93:3678–3684.
157. Liu C, Tadayoni BM, Bourret LA, Mattocks KM, Derr SM, Widdison WC, Kedersha NL, Ariniello PD, Goldmacher VS, Lambert JM, Blättler WA, Chari RVJ. Eradication of large colon tumor xenografts by targeted delivery of maytansinoids. Proc Natl Acad Sci USA 1996; 93:8618–8623.
158. Merchant AM, Zhu Z, Yuan JQ, Goddard A, Adams CW, Presta LG, Carter P. An efficient route to human bispecific IgG. Nat Biotechnol 1998; 16:677–681.
159. Sharma SK, Bagshawe KD, Melton RG, Sherwood RF. Human immune response to monoclonal antibody-enzyme conjugates in ADEPT pilot clinical trial. Cell Biophys 1992; 21:109–120.
160. Bosslet K, Czech J, Hoffmann D. Tumor-selective prodrug activation by fusion protein-mediated catalysis. Cancer Res 1994; 54:2151–2159.
161. Smith GK, Banks S, Blumenkopf TA, Cory M, Humphreys J, Laethem RM, Miller J, Moxham CP, Mullin R, Ray PH, Walton LM, Wolfe LA, 3rd. Toward antibody-directed enzyme prodrug therapy with the T268G mutant of human carboxypeptidase A1 and novel in vivo stable prodrugs of methotrexate. J Biol Chem 1997; 272:15804–15816.
162. Wolfe LA, 3rd, Mullin RJ, Laethem R, Blumenkopf TA, Cory M, Miller JF, Keith BR, Humphreys J, Smith GK. Antibody-directed enzyme prodrug therapy with the T268G mutant of human carboxypeptidase A1: in vitro and in vivo studies with prodrugs of methotrexate and the thymidylate synthase inhibitors GW1031 and GW1843. Bioconjug Chem 1999; 10:38–48.
163. Wu AM. Tools for pretargeted radioimmunotherapy. Cancer Biother Radiopharm 2001; 16:103–108.
164. Cremonesi M, Ferrari M, Chinol M, Stabin MG, Grana C, Prisco G, Robertson C, Tosi G, Paganelli G. Three-step radioimmunotherapy with yttrium-90 biotin:

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

dosimetry and pharmacokinetics in cancer patients. Eur J Nuc Med 1999; 26:110–120.
165. Paganelli G, Orecchia R, Jereczek-Fossa B, Grana C, Cremonesi M, De Braud F, Tradati N, Chinol M. Combined treatment of advanced oropharyngeal cancer with external radiotherapy and three-step radioimmunotherapy. Eur J Nuc Med 1998; 25:1336–1339.
166. Chinol M, Casalini P, Maggiolo M, Canevari S, Omodeo ES, Caliceti P, Veronese FM, Cremonesi M, Chiolerio F, Nardone E, Siccardi AG, Paganelli G. Biochemical modifications of avidin improve pharmacokinetics and biodistribution, and reduce immunogenicity. Br J Cancer 1998; 78:189–197.
167. Lasic DD, Papahadjopoulos D. Liposomes revisited. Science 1995; 267:1275–1276.
168. Kirpotin D, Park JW, Hong K, Zalipsky S, Li WL, Carter P, Benz CC, Papahadjopoulos D. Sterically stabilized anti-HER2 immunoliposomes: design and targeting to human breast cancer cells in vitro. Biochemistry 1997; 36:66–75.
169. Park JW, Hong K, Carter P, Asgari H, Guo LY, Keller GA, Wirth C, Shalaby R, Kotts C, Wood WI. Development of anti-p185^{HER2} immunoliposomes for cancer therapy. Proc Natl Acad Sci USA 1995; 92:1327–1331.
170. Park JW, Hong K, Kirpotin DB, Meyer O, Papahadjopoulos D, Benz CC. Anti-HER2 immunoliposomes for targeted therapy of human tumors. Cancer Lett 1997; 118:153–160.
171. Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, Jardieu PM. Humanization of an antibody directed against IgE. J Immunol 1993; 151:2623–2632.
172. Ädelroth E, Rak S, Haahtela T, Aasand G, Rosenhall L, Zetterstrom O, Byrne A, Champain K, Thirlwell J, Della Cioppa G, Sandström T. Recombinant humanized mAb-E25, an anti-IgE mAb, in birch pollen-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2000; 106:253–259.
173. Milgrom H, Fick RB, Jr., Su JQ, Reimann JD, Bush RK, Watrous ML, Metzger WJ. Treatment of allergic asthma with monoclonal anti-IgE antibody. N Engl J Med 1999; 341:1966–1973.
174. Bernstein JA. Anti-IgE reduces the requirement for inhaled corticosteroid treatment in children with moderate-severe asthma. Chest 2000; 118(suppl):100s.
175. Metzger JW. Anti-IgE reduces the requirement for inhaled corticosteroid treatment in patients with moderate-severe asthma. Chest 2000; 118(suppl):100s.
176. Solèr M, Matz J, Townley RG, Buhl R, O'Brien J, Fox HG, Thirlwell J, Rohane P, Fick R, Jr., Gupta N. RhuMAb-E25, a novel therapy for the treatment of allergic asthma (AA). Eur Respir J 2000; 16(suppl 31):10s.
177. Solèr M, Buhl R, Bensch G, Noga O, O'Brien J, Champain K, Fox H, Thirlwell J, Rohane R, Gupta N, Fick R, Della Cioppa G. Omalizumab (Xolair®, rhuMAb-E25) treatment reduces inhaled corticosteroid use in moderate/severe allergic asthma. Am J Resp Crit Care Med 2001; 163:A858.
178. Eigenbrot C, Randal M, Presta L, Carter P, Kossiakoff AA. X-ray structures of the antigen-binding domains from three variants of humanized anti-p185^{HER2} antibody 4D5 and comparison with molecular modeling. J Mol Biol 1993; 229:969–995.

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179. Pharmaceutical Research and Manufacturers of America. New Medicines in Development-Biotechnology: A 2000 Survey. Washington, DC: 2000, PhRMA.
180. Pharmaceutical Research and Manufacturers of America. New Medicines in Development for Cancer: A 2001 Survey. Washington, DC: 2001, PhRMA.
181. Nustad K, Bast RCJ, O'Brien TJ, Nilsson O, Seguin P, Suresh MR, Saga T, Nozawa S, Børmer OP, de Bruijn HWA, Nap M, Vitali A, Gadnell M, Clark J, Shigemasa K, Karlsson B, Kreutz FT, Jette D, Sakahara H, Endo K, Paus E, Warren D, Hammarström S, Kenemans P, Hilgers J. Specificity and affinity of 26 monoclonal antibodies against the CA125 antigen: first report from the ISOBM TD-1 workshop. Tumor Biol 1996; 17:196–219.
182. Hornick JL, Sharifi J, Khawli LA, Hu P, Bai WG, Alauddin M, Mizokami M, Epstein AL. Single amino acid substitution in the Fc region of chimeric TNT-3 antibody accelerates clearance and improves immunoscintigraphy of solid tumors. J Nucl Med 2000; 1:355–362.
183. Yang X-D, Jia X-C, Corvalan JRF, Wang P, David CG. Potent anti-tumor activity of ABX-EGF, a fully human monoclonal antibody to the epidermal growth factor receptor. Clin Cancer Res 1999; 5(suppl):85a.
184. Anderson D, Chambers K, Hanna N, Leonard J, Reff M, Newman R, Baldoni J, Dunleavy D, Reddy M, Sweet R, Truneh A. A primatized MAb to human CD4 causes receptor modulation, without marked reduction in CD4+ T cells in chimpanzees: in vitro and in vivo characterization of a MAb (IDEC-CE9.1) to human CD4. Clin Immunol Immunopath 1997; 84:73–84.
185. Yocum DE, Solinger AM, Tesser J, Gluck O, Cornett M, O'Sullivan F, Nordensson K, Dallaire B, Shen CD, Lipani J. Clinical and immunologic effects of a PRIMATIZED anti-CD4 monoclonal antibody in active rheumatoid arthritis: results of a phase I, single dose, dose escalating trial. J Rheumatol 1998; 25:1257–1262.

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