Transfusion Medicine

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THIRD EDITION

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Contents

Preface

It is a pleasure and an honor to prepare this third edition of *Transfusion Medicine*. It is a pleasure because of exciting developments that have occurred since the last edition and an honor because the previous editions have been sufficiently popular to warrant this new one. A wonderful benefit of writing another edition is the perspective it provides on changes in our field. For instance, there is little new discussion on leukodepletion and not as much new on hematologic growth factors in transfusion medicine as I expected. The biologic mechanism of transfusion-related immune modulation (TRIM) is still not understood, nor is the clinical effect, if any, of long stored red cells. Transfusion-related acute lung injury (TRALI) is still a problem, although apparently decreased by the use of plasma from male donors. The platelet transfusion trigger has been settled at 10,000 and the red cell transfusion trigger is lower than in the past, but there is still no physiologic key to red cell transfusion decisions. Quality has not fallen out of vogue but is less visible because it has taken its rightful place in operations. Apheresis technology continues to improve, enabling the collection of multiple combinations of components directly from the donor, and thus progressing toward elimination of the component laboratory. The technology is widely available to detect the genes for many red cell antigens, and it will be interesting to see how this is incorporated into practice when the next edition of this book is prepared. Production of novel cellular products is increasing, and these are used to treat conditions such as hematologic malignancies, immune diseases, myocardial damage, peripheral vascular disease, and to facilitate engraftment of stem cells or solid organs. The first novel cellular therapy product is now commercially available. While we have been extremely successful in reducing the transfusion-related risks of traditional agents such as HIV, HBV, HCV, and syphilis, this raises visibility of remaining risks such as Chagas' disease, babesiosis, dengue, and bacterial contamination along with short-lived concern about the XMRV agent that did not prove to be a clinical problem. However, these infectious agents illustrate that the approach to blood safety that has been so effective for the last 30 years may not cope with the future. The logical approach going forward is pathogen inactivation, which is being widely adopted for plasma and platelets outside the United States. If methods to treat red cells prove successful, this will allow comprehensive changes in testing and irradiation practices, and if the process can be done at low cost, there are exciting possibilities for the developing world where transmissible disease remains a major problem.

The book is intended to be comprehensive and extensively referenced yet easy to read. It should be helpful to those with a first exposure to transfusion medicine, such as residents and fellows, but also valuable for those doing transfusion medicine full-time or supervising hospital blood bank laboratories on a part-time basis. I hope it is especially valuable for physicians such as hematologists, surgeons, and anesthesiologists who use blood in their practice.

While this is a single-author book, it cannot be prepared alone. Physicians at the University of Minnesota, North Central Region of the American Red Cross, and Memorial Blood Centers of Minnesota have contributed more than they know by my involvement in that wonderful group and of what I have learned from those interactions. My friend and former fellow, S. Yoon Choo, prepared the exceptional chapter on human lymphocyte antigens (HLA). Colleagues from throughout the United States and the world have enriched my understanding that thus contributes to this book. Then there is the hard work of organizing, word processing, and reference searching that has been provided by my long-time assistant, Penny Milne, and, more recently, Erinn Riley and Ashley Delisle.

This book would not be possible without all of these people, and I am indebted to them for their friendship, support, and assistance.

I hope you enjoy this edition.

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1 History

1.1 Ancient times

For centuries, blood has been considered to have mystical properties and has been associated with vitality. In ancient times, bathing in or drinking the blood of the strong was thought to invigorate the weak. For instance, among Ancient Romans it was customary to rush into the arena to drink the blood of dying gladiators [1]; among others, to drink or bathe in blood was thought to cure a variety of ailments [2]. Bleeding was practiced to let out bad blood and restore the balance of humors, thus hopefully returning the patient to health.

It is not known when and by whom the idea of transfusing blood was developed. It is said that the first transfusion was given to Pope Innocent VIII in 1492. According to this legend, the Pope was given the blood of three boys, whose lives were thus sacrificed in vain [1, 3] because the attempts did not save the Pope. In another version of the story, the blood was intended to be used in a tonic for the Pope, which he refused, thus sparing the boys' lives [2].

1.2 The period 1500–1700

Others to whom the idea for blood transfusion is attributed include Hieronymus Cardanus (1505–1576) and Magnus Pegelius. Little is known about Cardanus, but Pegelius was a professor at Rostock, Germany, who supposedly published a book describing the idea and theory of transfusion [1]. It can be substantiated that Andreas Libavius (1546–1616) proposed blood transfusion when in 1615 he wrote:

> Let there be a young man, robust, full of spirituous blood, and also an old man, thin, emaciated, his strength exhausted, hardly able to retain his soul. Let the performer of the operation have two silver tubes fitting into each other. Let him enter the artery of the young man, and put into it one of the tubes, fastening it in. Let him immediately open the artery of the old man and put the female tube into it, and then the two tubes being joined together, the hot and spirituous blood of the young man will pour into the old one as it were from a fountain of life, and all of this weakness will be dispelled [1].

Despite these possibilities, it also seems unlikely that the concept of transfusing blood could have developed before William Harvey's description of the circulation in 1616. Despite Harvey's description of the circulatory system, there is no evidence that he considered blood transfusion. However, the concept of the "circulation" may have preceded Harvey's publication. For instance, Andrea Cesalpino (1519–1603), an Italian, used the expression "circulation" and proposed that fine vessels (capillaries) connected the arterial and venous systems [1, 4].

A number of the major developments that led to the beginning of blood transfusion occurred during the mid-1600s [1]. In 1656, Christopher Wren, assisted by Robert Boyle, developed techniques to isolate veins in dogs and carried out many studies of the effects of injecting substances into the dogs. It is not clear whether Wren ever carried out blood transfusion between animals. The first successful transfusion from one animal to another probably was done by Richard Lower [1, 5, 6]. Lower demonstrated at Oxford the bleeding of a dog until its strength was nearly gone but revitalized the previously moribund dog by exchange transfusion using blood from two other dogs, resulting in the death of the donor animals [6].

Subsequently, a controversy developed over who had first done a transfusion. In 1669, Lower contended that he had published the results of transfusion in the Philosophical Transactions of the Royal Society in December 1666. In 1667, Jean Denis of France described his experiments in animals and applied the technique to man, which Lower had accomplished only in animals. Others mentioned as possibly having carried out animal-to-animal transfusions about this time are Johann-Daniel Major of Cologne, Johann-Sigmund Elsholtz of Berlin, don Robert de Gabets (a monk) in France, Claude Tardy of Paris, and Cassini and Griffone in Italy [1].

Denis apparently was a brilliant young professor of philosophy and mathematics at Montpellier and physician to Louis XIV. In 1667, Denis carried out what is believed to be the first transfusion of animal (lamb's) blood to a human. A 15-year-old boy with a long-standing fever, who had been bled multiple times, received about 9 ounces of blood from the carotid artery of a lamb connected to the boy's arm vein. Following the transfusion, the boy changed from a stuporous condition to a clear and smiling countenance. During the next several months, Denis may have given transfusions to three other individuals [1]. The second patient, Antoine Mauroy, was an active 34-year-old who spent some of his time carousing in Paris. It was thought that blood from a gentle calf might dampen Mauroy's spirits. On December 19, 1667, he received with no untoward effects 5 or 6 ounces of blood from the femoral artery of a calf. Several days later, the procedure was repeated. During the second transfusion, Mauroy experienced pain in the arm receiving the blood, vomiting, increased pulse, a nosebleed, pressure in the chest, and pain over the kidneys; the next day he passed black urine. This is probably the first reported hemolytic transfusion reaction. Mauroy died about 2 months later without further transfusions. Reportedly, members of the Faculty of Medicine who were opposed to transfusion and hated Denis bribed Mauroy's wife to state that he had died during the transfusion [1]. Denis

was tried for manslaughter but was exonerated. It was later revealed that Mauroy's wife had been poisoning him with arsenic and that was the actual cause of his death [7]. Also in late 1667, Lower performed a human transfusion before the Royal Society in England. The man received 9–10 ounces of blood from the artery of a sheep and was said to have "found himself very well" afterward [1]. However, the death of Mauroy was used by Denis' enemies as an excuse to issue an edict in 1668 that banned the practice of transfusion unless the approval of the Faculty of Medicine in Paris was obtained. This series of events led to the discontinuation of transfusion experiments, but more importantly to the abandonment of the study of the physiology of circulation for approximately 150 years [1].

1.3 The 1800s

Interest in transfusion was revived during the early 1800s, primarily by James Blundell, a British obstetrician who believed it would be helpful in treating postpartum hemorrhage [8]. Blundell carried out animal experiments and avoided the error of using animal blood because of the advice of a colleague, Dr. John Leacock. Blundell reported to the Medico-Chirurgical Society of London on December 22, 1818, the first human-to-human transfusion. It is not clear whether the transfusions given by Blundell were ever successful clinically [1]. However, Blundell's contributions were very substantial. Unfortunately, his warnings about the dangers of transfusing animal blood into humans were not generally heeded.

Dr. Andrei Wolff carried out a human-to-human transfusion in St. Petersburgh, Russia, in 1832 having learned of blood transfusion from Dr. Blundell on a previous visit to London [9]. There is no evidence of additional transfusion in Russia until the 1920s when a transfusion institute was established in Moscow.

Key work in understanding the problems of using animal blood for human transfusions was provided by Ponfick and Landois [1]. They observed residues of lysed erythrocytes in the autopsy serum of a patient who died following transfusion of animal blood. They also noted pulmonary and serosal hemorrhages, enlarged kidneys, congested hemorrhagic livers, and bloody urine due to hemoglobinuria and not hematuria when sheep's blood was transfused to dogs, cats, or rabbits. Landois observed that human red cells would lyse when mixed in vitro with the sera of other animals. Thus, evidence mounted that interspecies transfusion was likely to cause severe problems in the recipient.

1.4 First transfusions in the United States

In the United States, transfusions were first used in the mid-1800s, but it is not clear where they were first performed. They may have been done in New Orleans in about 1854 [2]. During the Civil War, the major cause of death was hemorrhage [10]. However, at that time blood transfusion was not developed, and it appears to have been used in only two to four

patients [2]. Two cases are described by Kuhns [10]. One was transfused at Louisville and one at Alexandria within about 10 days of each other. There is no evidence that the procedures were jointly planned or that the physicians involved communicated about them. In both cases, the patients improved following the transfusions [10].

1.5 The discovery of blood groups

The accumulating experiences began to make it clear that transfusions should be performed only between members of the same species. However, even within species transfusions could sometimes be associated with severe complications. Because of this, and despite the experiences during the Civil War, few transfusions were carried out during the last half of the 1800s. The discovery of blood groups by Landsteiner opened a new wave of transfusion activity. It had been known that the blood of some individuals caused agglutination of the red cells of others, but the significance of this was not appreciated until Landsteiner in 1900 reported his studies of 22 individuals in his laboratory. He showed that the reactions of different combinations of cells and sera formed patterns and these patterns indicated three blood groups [11]. He named these blood groups A, B, and C (which later became group O). Apparently none of the staff of Landsteiner's laboratory had the less common group AB, but soon this blood group was reported by the Austrian investigators Decastello and Sturli [1]. Soon thereafter, several other nomenclature systems were proposed, and the American Medical Association convened a committee of experts, who recommended a numerical nomenclature system [12] that never gained widespread use [11]. Others later demonstrated that the blood groups were inherited as independent Mendelian dominants and that the phenotypes were determined by three allelic genes. Hektoen of Chicago first advocated the use of blood grouping to select donors and recipients and to carry out transfusion [13], but it was Ottenberg who put the theory into practice [14]. These activities are the basis for the widely held belief that blood banking in the United States had its origins in Chicago.

1.6 Anticoagulation

Another factor that inhibited the use of transfusions during the late 1800s was blood clotting. Because of the inability to prevent clotting, most transfusions were given by direct methods. There were many devices for direct donor-to-recipient transfusion that incorporated valves, syringes, and tubing to connect the veins of donor and recipient [15].

   Although there were many attempts to find a suitable anticoagulant, the following remarks must be prefaced by Greenwalt's statement that "none of them could have been satisfactory or else the history of blood transfusion would have had a fast course" [1]. Two French chemists, Prevost and Dumas, found a method to defibrinate blood and observed that such blood was effective in animal transfusions [1]. Substances tested for

anticoagulation of human blood include ammonium sulfate, sodium phosphate, sodium bicarbonate, ammonium oxalate and arsphenamine, sodium iodide, and sodium sulfate [16, 17]. The delays in developing methods to anticoagulate blood for transfusion are interesting because it was known in the late 1800s that calcium was involved in blood clotting and that blood could be anticoagulated by the addition of oxalic acid. Citrates were used for laboratory experiments by physiologists and by 1915 several papers had been published describing the use of sodium citrate for anticoagulation for transfusions [1]. It is not clear who first used citrated blood for transfusion [1]. It could have been Lewisohn [18], Hustin, or Weil [19]. In 1955, Lewisohn received the Landsteiner award from the American Association of Blood Banks for his work in the anticoagulation of blood for transfusion.

1.7 Modern blood banking and blood banks

Major stimuli for developments in blood transfusion have come from wars. During World War I, sodium citrate was the only substance used as an anticoagulant. Dr. Oswald Robertson of the U.S. Army Medical Corps devised a blood collection bottle and administration set similar to those used several decades later [1] and transfused several patients with preserved blood [20].

Between World Wars I and II, there was increasing interest in developing methods to store blood in anticipation of rather than response to need. It has been suggested that the first "bank" where a stock of blood was maintained may have been in Leningrad in 1932 [1, 2]. A blood bank was established in Barcelona in 1936 because of the need for blood during the Spanish Civil War [21]. In the United States, credit for the establishment of the first blood bank for the storage of refrigerated blood for transfusion is usually given to Bernard Fantus at the Cook County Hospital in Chicago [22]. The blood was collected in sodium citrate and so it could be stored for only a few days.

1.8 Cadaver blood

Cadavers served as another source of blood during the 1930s and later. Most of this work was done by Yudin [23] in the USSR. Following death, the blood was allowed to clot, but the clots lysed by normally appearing fibrinolytic enzymes, leaving liquid defibrinated blood.

The use of cadaver blood in the Soviet Union received much publicity and was believed by many to be the major source of transfusion blood there. Actually, not many more than 40,000 200-mL units were used, and most of them at Yudin's Institute [1]. In 1967, the procedure was quite complicated, involving the use of an operating room, a well-trained staff, and extensive laboratory studies. This was never a practical or extensive source of blood.

1.9 The Rh blood group system and prevention of Rh immunization

In 1939, Levine, Newark, and Stetson published in less than two pages in the *Journal of the American Medical Association* [24] their landmark article, a case report, describing hemolytic disease of the newborn (HDN) and the discovery of the blood group that later became known as the Rh system. A woman who delivered a stillborn infant received a transfusion of red cells from her husband because of intrapartum and postpartum hemorrhage. Following the transfusion, she had a severe reaction but did not react to subsequent transfusions from other donors. The woman's serum reacted against her husband's red cells but not against the cells of the other donors. Levine, Newark, and Stetson postulated that the mother had become immunized by the fetus, who had inherited a trait from the father that the mother lacked. In a later report they postulated that the antibody found in the mother and subsequently in many other patients was the same as the antibody Landsteiner and Wiener prepared by immunizing Rhesus monkeys [25]. This also began a long debate over credit for discovery of the Rh system.

During the early 1900s, immunologic studies had established that active immunization could be prevented by the presence of passive antibody. This strategy was applied to the prevention of Rh immunization in the early 1960s in New York and England at about the same time [26, 27]. Subjects were protected from Rh immunization if they were given either Rh-positive red cells coated with anti-Rh or anti-Rh followed by Rh-positive red cells. Subsequent studies established that administration of anti-Rh in the form of Rh immune globulin could prevent Rh immunization and thus almost eliminate HDN. Currently, control of HDN is a public health measure similar to ensuring proper immunization programs for susceptible persons.

1.10 Coombs and antiglobulin serum

In 1908, Moreschi [28] is said to have described the antiglobulin reaction. The potential applicability of this in the detection of human blood groups was not appreciated until 1945 when Coombs, Mourant, and Race [29] published their work on studies of the use of rabbit antibodies against human IgG to detect IgG-coated red cells. Red cells were incubated with human sera containing antibodies against red cell antigens, washed, and the rabbit antihuman sera used to demonstrate the presence of bound IgG by causing agglutination of the red cells. The availability of antihuman globulin serum made it possible to detect IgG red cell antibodies when the antibody did not cause direct agglutination of the cells. Thus, red cells coated with anti-IgG red cell antibodies could be easily detected, and the era of antibody screening and crossmatching was born. This greatly improved the safety of blood transfusion and also led to the discovery of many red cell antigens and blood groups.

1.11 Plasma and the blood program during World War II

Techniques for collection, storage, and transfusion of whole blood were not well developed during the 1930s. The outbreak of World War II added further impetus to the development of methods to store blood for periods longer than a few days. Although the method of blood anticoagulation was known by the mid-1920s, red blood cells hemolyzed after storage in sodium citrate for 1 week. This limitation also slowed the development of blood transfusion. Although it was also known that the hemolysis could be prevented by the addition of dextrose, the practical value of this important observation was not recognized for over a quarter of a century. Anticoagulant preservative solutions were developed by Mollison in Great Britain [30]. However, when the glucose–citrate mixtures were autoclaved, the glucose caramelized, changing the color of the solution to various shades of brown. The addition of citric acid eliminated this problem and also extended the storage time of blood to 21 days. The advance of World War II also brought an understanding of the value of plasma in patients with shock [31, 32]. In the early 1940s, Edwin J. Cohn, PhD, a Harvard biochemist, developed methods for the continuous flow separation of large volumes of plasma proteins [33, 34]. This made possible during World War II the introduction of liquid and lyophilized plasma and human albumin as the first-line management of shock. Initial work using plasma for transfusion was carried out by John Elliott [31, 32]. This combination of technological and medical developments made it possible for Charles R. Drew to develop the "Plasma for Britain" program [35].

1.12 Plastic bags and blood components

One of the next major developments in blood banking was the discovery and patenting of the plastic blood container by Carl Walter in 1950. This made possible the separation of whole blood and the creation of blood component therapy. Dr. Walter's invention was commercialized by the Baxter Corporation. Fenwal division that later became a freestanding company. The "-wal" of Fenwal represents Dr. Walter's name. The impact of the introduction of multiple connected plastic containers and the separation of whole blood into its components also began to generate enormous amounts of recovered plasma, which made possible the development of large-scale use of coagulation factor VIII concentrates.

1.13 Cryoprecipitate and factor VIII

In 1965, Dr. Judith Pool reported that if fresh frozen plasma (FFP) was allowed to thaw at refrigerator temperatures, precipitate remained that contained most of the coagulation factor VIII from the original FFP [36].

This made it possible for the first time to administer large doses of factor VIII in a concentrated form to hemophiliacs and opened an era in which the bleeding diathesis could be effectively managed. A few years later, reports began to appear describing the use of a concentrated factor VIII prepared using the plasma fractionation technique developed by Edwin Cohn [33]. This further simplified the management of hemophilia because the ability to store the factor VIII concentrates in home refrigerators enabled the development of home treatment programs involving prophylactic or immediate self-administration of factor VIII.

1.14 Red cell preservation

The role of 2,3-diphosphoglycerate in oxygen transport by red cells was discovered in the mid 1960s [37, 38]. It had been known previously that this compound was better maintained at higher pH, while adenosine triphosphate (ATP), which appeared to be involved in red cell survival, was maintained better at a lower pH. The addition of adenine was shown to improve ATP maintenance and prolong red cell survival and storage for transfusion [39]. The next major advance in red cell preservation was the development of preservative solutions designed to be added after removal of most of the original anticoagulated plasma, thus further extending the storage period of red cells [4, 40].

1.15 Leukocyte antigens and antibodies

In 1926, Doan described the sera of some individuals that caused agglutination of the leukocytes from others [41]. Subsequent studies established the presence of leukocyte antibodies, the presence of these antibodies in the sera of polytransfused patients, the occurrence of white cell agglutinins in response to fetomaternal immunization, and the alloimmune and autoimmune specificities associated with these antibodies. These studies, along with studies of the murine histocompatibility system, led to the description of the major histocompatibility system (human lymphocyte antigens (HLA)) [42] in humans and the understanding that there are separate antigenic specificities limited to neutrophils as well [43]. These studies also defined the causative role of leukocytes in febrile nonhemolytic transfusion reactions [44]. Strategies were sought to prevent these reactions by removing the leukocytes from blood [45, 46], one of the first methods being reported by Fleming [46], the discoverer of penicillin.

1.16 Platelet collection, storage, and transfusion

The relationship between bleeding and thrombocytopenia had been known for some time, but the development of the plastic bag system for blood collection made platelets available for transfusion. Several years of

work by many investigators—predominantly at the National Cancer Institute during the 1960s—developed the methods for preparing platelets and established that platelet transfusion to thrombocytopenic patients reduced mortality from hemorrhage [47]. Initially, platelets had to be transfused within a few hours after the whole blood was collected, and thus large-scale application in the general medical care setting was impractical. The seminal report by Murphy and Garner [48] showing that room temperature allowed platelets to be stored for several days revolutionized platelet transfusion therapy.

1.17 Apheresis

Plastic bags were used to remove whole blood, separate the plasma from the red cells, retain the plasma, and return the red cells, thus making it possible to obtain substantial amounts of plasma from one donor [49]. This initiated the concept of attempting to obtain only selected portions of whole blood in order to collect larger amounts of plasma or cells. The centrifuge developed by Cohn for plasma fractionation was modified by Jack Latham and became a semiautomated system for plasmapheresis [50] and subsequently was used for platelet collection as well [51, 52]. At the National Institutes of Health Clinical Center, an IBM engineer worked with hematologists to develop a centrifuge that enabled collection of platelets or granulocytes from a continuous flow of blood through the instrument [53, 54]. Later versions of these instruments have become widely used for plateletpheresis and leukopheresis.

1.18 Granulocyte transfusions

As the benefits of platelet transfusion for thrombocytopenic patients were recognized, interest developed in using the same strategy to provide granulocyte transfusion to treat infection in neutropenic patients. Initial attempts involved obtaining granulocytes from patients with chronic myelogenous leukemia (CML) [55, 56]. Transfusion of these cells had clinical benefits [57], and this led to a decade of effort to develop methods to obtain granulocytes from normal donors [58]. At best, these methods produced only modest doses of granulocytes; improvements in antibiotics and general patient care have supplanted the need for granulocyte transfusions except in very limited circumstances (see Chapter 12).

1.19 Summary

Blood banking and transfusion medicine developed slowly during the 1950s but much more rapidly between the 1960s and the 1980s. Some of the important advances mentioned here were understanding blood groups and the identification of hundreds of specific red cell antigens; the development of the plastic bag system for blood collection and separation;

plasma fractionation for the production of blood derivatives, especially factor VIII; improved red cell preservation; platelet preservation and transfusion; understanding hemolytic and febrile transfusion reactions; expanded testing for transmissible diseases; and the recognition of leukocyte and platelet antigen systems. Blood collection and storage is now a complex process operated much like the manufacture of a pharmaceutical. Transfusion medicine is now the complex, sophisticated medical–technical discipline that makes possible many modern medical therapies.

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2 The Blood Supply

Blood transfusion occurs in all parts of the world, but the availability, quality, and safety of the blood depends on the general status of medical care in that area. Approximately, 75–90 million units of blood are collected annually worldwide [1]. The amount of blood collected in relation to the population ranges from 40 donations per 1,000 population in industrialized countries to 10 donations per 1,000 in developing countries and 3 donations per 1,000 in the least developed countries [2]. Thus, there is a concentration of blood transfusion in industrialized countries, with 20% of the world's population receiving approximately 55% of the world blood supply [1]. Lack of blood is a major problem in many parts of the world. For instance, about 34% of maternal and 15% of childhood mortality in Sub-Saharan Africa are due to malaria, when appropriate transfusion therapy is not available [3]. It is generally thought that blood services are best provided if there is a national, or at least regional, organization [4–6]. It is important that the government make a commitment to the nation's blood supply (Table 2.1). The blood supply may be provided by individual hospitals, private blood banks, the Red Cross, Ministries of Health, or some other part of the national government. The number of units of blood collected at individual centers can range from a few hundreds to thousands per year and there may be extensive or very little coordination and standardization. The adoption of a national blood policy is recommended along with establishing a national organization [6–8]. This has been achieved in the developed world where virtually all countries operate a national blood supply system as part of their public health structure as recommended by World Health Organization (WHO) [6,9]. Centralization of blood supply systems has also begun in Latin America [4] and Sub-Saharan Africa [10–15]. The United States is essentially the only developed country without a single unified national blood supply organization.

   Although great progress has been made in establishing national or centralized blood transfusion services, some blood is still collected without national control or organization. In many parts of the world, there is little or no organized donor recruitment system and so the blood supply fluctuates. In 38 countries, more than 75% of blood is donated by friends

Table 2.1 Key elements of a nationally coordinated blood transfusion service[a].

Government commitment
A national blood policy
Formation or designation with responsibility to operate the program
Appointment of a suitable director
Appointment of qualified staff
Development of partnerships with appropriate NGOs
National guidelines for the clinical use of blood
Identification of low risk donor populations and development of strategies to promote blood
Donation
Education programs for physicians, nurses, and other appropriate staff regarding transfusion
Therapy
Systems for donor notification and counseling

[a]Blood transfusion safety: voluntary blood donation, national blood transfusion services, and safe and appropriate use; WHO web site programs and projects.

or relatives of patients who are transfusion recipients [Table 2.2) [17–19]. Although these donors are considered to be volunteers, they may be donating under family pressure or they may be individuals unknown to the family who have been paid to donate blood. This is unfortunate because the risk of transfusion-transmitted infection from first-time [2, 20] and paid [21] donors is much higher than from volunteers, although not all agree [22] (Chapter 3). These risks are further accentuated by the lack of testing of donor blood for transfusion-transmissible diseases that sometimes occurs in developing and least developed countries (Table 2.2). This, combined with the use of replacement or paid donors and the low rates of repeat blood donors with their lower rate of positive tests for transfusion-transmissible diseases, leads to a major concern about blood safety in developing and least developed countries [16, 23, 24]. As many as 13 million of the 75–90 million units collected annually are not tested for at least one of human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), or syphilis [19]. This is because of a shortage of trained staff, unavailability or poor quality of test kits, or infrastructure breakdowns. Sometimes transmissible disease testing is not done because the need is so urgent that the blood must be transfused immediately after it is collected. The cost of transmissible disease testing is also problematic because it may approach the annual per capita expenditure for all of health care in some countries [25]. While there is a worldwide blood shortage and testing for transmissible diseases is not done on a substantial portion of the world's blood supply, impressive progress has been made in establishing testing systems, increasing blood collections, standardizing operations, and increasing the availability of safe blood [7, 9–20, 22–25].

In contrast, the US blood supply is provided by many different organizations with different organizational structures and philosophies. These organizations function rather effectively to meet the nation's blood needs and thus are referred to here as the US blood supply system,

Table 2.2 Activities related to blood availability and safety in different countries[a].

	Donor testing for			All volunteer donors	Some replacement donors	Some paid donors	% Repeat donors
	HIV	HBV	Syphilis				
Developed	100	100	94	85	20	5	88
Developing	66	72	71	15	80	25	47
Least developed	46	35	48	7	93	25	20

[a]Data summarized from Gibbs WN, Corcoran P. Blood safety in developing countries. Vox Sang 1994; 67:377–381.

although they are not really a unified system. A comprehensive report on the nation's blood collection system was prepared several years ago by the Office of Technology Assessment [26]. Although time has passed and some details are different today, the general description of the blood collection and supply system in that report is still valid.

Blood can be collected in two ways and this has resulted in the development of two different kinds of systems in the United States. One involves the cellular elements and plasma obtained from whole blood or by cytapheresis. Most of that supply of blood and components is provided by nonprofit community blood centers; almost half is from the American Red Cross. Hospitals collect about 10% of the nation's blood supply [27]. These cellular products and unprocessed plasma are collected by blood banks and used directly for transfusion prepared by blood centers.

The other blood collection system involves large-scale collection of plasma by plasmapheresis for the subsequent manufacture of plasma derivatives. This is done by a separate set of for-profit organizations and almost all of this plasma comes from paid donors. This plasma is manufactured into plasma derivatives such as albumin, coagulation-factor concentrates, or intravenous immunoglobulin (IVIG) and these are sold in the national and international market.

2.2 The blood collection system

Whole blood is collected by venipuncture from healthy adults into plastic bags containing a liquid anticoagulant preservative solution. The whole blood is separated into red blood cells, platelet concentrate, and fresh frozen plasma (see Chapter 5). The fresh frozen plasma can be (a) used for transfusion, (b) further processed into cryoprecipitate (to be used for transfusion) and cryoprecipitate-poor plasma (which serves as a raw material for further manufacture of plasma derivatives), or (c) provided as a source of raw material for subsequent manufacture of plasma derivatives as described below. Blood banks make many modifications to these components to obtain blood products that will be effective for specific purposes. Descriptions of these various blood components and their

medical uses are provided throughout this book. A complete list of components that can be produced from whole blood and are licensed by the US Food and Drug Administration (FDA) is provided in Chapter 5. In addition, blood banks distribute many of the plasma derivative products as part of their total supply program for transfusion medicine therapy, but most of these other plasma products are manufactured by plasma derivative companies and distributed to hospital pharmacies. Blood centers also produce platelets and granulocytes by apheresis (see Chapter 7) in which the cells of interest are removed in a blood cell separator and the remaining blood is returned to the donor.

The US blood collection system is heterogeneous because blood centers developed for a variety of reasons mostly during the 1940s and 1950s. Some were continuations of blood collection activities initiated during World War II; others were civic or philanthropic activities, and some were formed by groups of hospitals to collect blood for their own needs. However, in the last 30 years, blood center activity has increased and most hospitals have stopped collecting blood so that currently about 90% of the US blood supply is collected by blood centers [27].

In most areas of the United States, there is only one local organization that collects blood. Blood centers are freestanding organizations, almost all of which are nonprofit. These centers are governed by a board of local volunteers; their sole or major function is to provide the community's blood supply. Each blood center collects blood in a reasonably contiguous area. The blood center may or may not supply the total needs of the hospitals in its area and may supply hospitals in other areas as well. The area covered by each center is determined by historical factors and is not developed according to any overall plan. Rather, local interests dictate whether, how, and what kind of community blood program is developed. There are a total of approximately 144 blood centers in the United States [27]. Approximately, 35 of these are operated by the American Red Cross (ARC) and the remainder are community blood centers.

Traditionally, blood centers had an organizational culture resembling the practice of medicine and operated somewhat like a clinical hospital laboratory [28, 29]. As a result of the HIV epidemic, concern about blood safety increased and blood supply organizations were subjected to political and media scrutiny. About this time, the regulatory environment also changed [30, 31]. As a result, the blood collection system in the United States underwent substantial change [28, 29, 31].

Major changes have been made in the medical criteria for selection of donors and in the laboratory testing of donated blood (see Chapters 4 and 8). In addition, more fundamental changes have been implemented in the nature of the organizations that make up the blood supply system. The organizations have adopted philosophies and organizational structures resembling those found in the pharmaceutical industry rather than the previous hospital laboratory and medical model. Modern quality assurance systems and good manufacturing practices [31] like those used in the pharmaceutical industry have been introduced. New computer systems now provide greater control over the manufacturing process [32]

and changed management structures deal with the new kinds of activities and philosophy. Blood centers and supply organizations are now operated using a very structured business and manufacturing philosophy, organization, and culture.

The ARC is the organization that collects about 6,000,000 units of whole blood annually or slightly less than half of the nation's blood supply. The ARC is a nonprofit, congressionally chartered (but not government sponsored or operated) organization that conducts programs supported by donated funds and/or cost recovery. The Red Cross operates through a network of regional centers. The blood is provided to hospitals and transfusion services, and the Red Cross charges a fee to cover the cost of collecting, testing, processing, storing, and shipping the blood. All non-ARC blood centers are community-based, nonprofit organizations that are members of America's Blood Centers, which accounts for slightly less than half of the nation's blood supply.

Blood banks that are part of hospitals usually collect blood only for use in that hospital and do not supply other hospitals. However, few, if any, hospitals collect enough blood to meet all their needs. They purchase some blood from a local or distant community blood center. Most hospitals in the United States do not collect any blood but acquire all of the blood they use from a community center. Of those that do collect blood, there are no good data available to define the proportion of their needs that they collect. This can be presumed to be quite variable.

2.3 Amount of blood collected

In 2006, 14,151,000 units of allogeneic whole blood, 335,000 (2.1%) units of autologous blood, and 70,000 (0.4%) units of directed donor blood were collected [27]. Thus, the total amount of blood collected in the United States was approximately 14,560,000 units. An additional 1,619,000 units of red cells (10%) were collected by apheresis giving a total of 16,174,000 units. Laboratory testing led to discard of 151,000 (0.9%) leaving a total of 16,023,000 units available for transfusion.

There have been several trends in the nation's blood supply since the 1970s, undoubtedly influenced by the AIDS epidemic. From 1988 to 1997, there was a 9.6% decrease in the amount of allogeneic blood collected [27]. The collection of autologous blood increased rapidly (23% annually) during the period 1988–1992; however, between 1992 and 1997, there was a large decrease (42%) in autologous blood collections [27]. Thus, the growth rate of blood collections experienced during the 1970s and early 1980s has slowed during the past few years. The total number of units collected in 2006 was 5.8% greater than in 2004 [27]. Autologous and directed donations continued a multi-year decline, decreasing by 26.9% and 40.1%, respectively, between 2004 and 2006. Collection of red cells by apheresis increased by 96.4% from 2004.

The excess of blood collected compared with that transfused is another indication of the adequacy of the blood supply. This excess was 10–12% from

1989 to 1994 [27] and remained substantial at 7.8% in 2006 [27]. It seems as if this excess should be adequate, although 6.9% of hospitals reported canceling elective surgery during 2006 due to shortage of blood [27].

About one-third of red cell units are used for surgery, one-third for hematology–oncology, and one-third for other medical indications. Most transfusions are done with considerable clinical urgency and only about 10% for nonurgent medical conditions or elective surgery [32a]. Approximately 8% of a national blood supply is used for patients in intensive care units. About 40% of those patients receive transfusions [32b]. In times of inventory shortage, conserving or postponing elective transfusions to medical patients conserves a larger proportion of the red cell supply than canceling major elective surgery [32c].

Blood component production

In 2006, 13,335,000 units of platelets (equivalent to one unit of whole blood) were produced [27]. This represents a 5.2% increase in the production of platelets between 2004 and 2006. Of these, 2,396,000 were produced from whole blood and 10,939,000 were produced by apheresis. Between 2004 and 2006, production of platelets by apheresis increased 19.4%, while whole blood-derived platelets decreased 43%. The shift to apheresis platelets continued with apheresis now accounting for about 82% of the platelet supply.

In 2006, 5,624,000 units of fresh frozen plasma [23% increase) and 1,197,000 units of cryoprecipitate (2.8% increase) were produced (27].

Nonutilization of donated blood [24]

During 2006, approximately 401,000 units of red blood cells or 2.4% of the total collected were lost, not used, or unaccounted for [27]. The nontransfusion rate for autologous blood was 32% and for directed-donor red cells about 1.2% because about 44% of directed donor units were placed in the general inventory. Of the nonred cell components, whole blood-derived platelets had a 22% outdate rate, apheresis platelets 15%, frozen plasma 20%, and cryoprecipitate 2.8% [27].

2.4 Blood inventory sharing systems

Certain areas of the United States are chronically unable to collect enough blood to meet their local transfusion needs. Some areas of the United States have been able to collect more blood than is needed locally and have provided these extra units to communities in need. The misalignment of blood use and blood collection is a long-standing phenomenon. Blood banks in metropolitan areas that serve large trauma, tertiary, and transplantation centers most frequently experience shortages of whole blood, components, and type-specific blood units. Blood supply to metropolitan areas is especially difficult if the local community blood center does not include a large rural population in their blood service area.

To protect themselves against constant shortages, these blood banks could no longer rely on the random availability of surplus units from their neighboring blood banks. Many blood banks participate in systems to exchange blood among themselves to alleviate shortages. Blood may be "exported" from areas of oversupply and "imported" into areas of shortage—a practice called "blood resource sharing." The lack of an adequate local blood supply and the need to import blood causes several difficulties, including possible unavailability of blood or components when needed, complex inventory management, technical disparities, emergency appeal-type donor recruitment, higher costs, decreased independence, and higher risk management costs. Blood resource sharing may also be used for financial reasons. Some blood centers import blood because they can obtain this blood less expensively than their own costs of production. Other blood centers export blood because the increased volume of collection helps to reduce their own average costs.

In the early 1960s, the American Association of Blood Banks (AABB) established a national clearinghouse so that blood could be moved nationally in response to need.

The concept of blood as a national resource has slowly gained favor. Despite the fact that there is not a unified blood banking system or a single national inventory or blood resource sharing system in the United States, blood banks have made major efforts to utilize blood from areas where it is available in excess. Today, a considerable amount of blood resource sharing occurs in the United States. A substantial proportion of blood collected by the American Red Cross is actually distributed to hospitals by a regional center different from the region where the blood was collected and the AABB operates the National Blood Exchange that coordinates the distribution of about 240,000 units of blood and components annually.

One of the major issues in blood resource sharing is the attitude of blood donors. In the only study focused on donors' attitudes about being asked to donate more blood than is needed by their local community [33], donors to several ARC blood centers indicated a willingness to donate for patients in other areas of the United States as long as their local blood needs were being met.

Exporting and importing blood centers

Some blood centers collect more than they need because besides assisting blood banks that experience shortages, collecting additional blood units may improve economies of scale and may help to reduce local fees for blood. In addition to establishing long-term agreements, some exporting centers have surplus blood units available to ship on an ad hoc basis. Depending on the point of shipment and the level of supply, blood purchased on an ad hoc basis may be more costly to the importing than blood acquired through long-term contractual agreements. While exporting centers assist immeasurably in helping to meet shortages in certain areas, the normal fluctuations that occur on both the supply and demand sides make a perfect balance very difficult to achieve. The exporting center may be able to provide blood units at a fee below that of

the local blood provider for a number of reasons. Hospitals may use the imported units first, holding the local supplier's units in inventory in the event of shortage and then returning the unused units to the local supplier for credit prior to outdate. The exporting center usually provides only routine units in the shipment but not special units such as rare blood types, cytomegalovirus-negative (CMV-negative) units, or irradiated units. The exporting center does not provide any ancillary services such as medical consultation, special testing, or reference laboratory services. The exporting center most frequently drop-ships in times of excess supply, which may create wide variations in the number of units that the local blood bank must supply. Finally, the exporting center may contract only with large hospitals in the community, where savings are realized as a result of high-volume shipments. While the availability of lower-cost blood may be appealing to the hospital, it increases the complexity and thus the costs of operating the local community blood center and overall may not be cost-effective. Some urban hospitals with specialty and emergency centers require blood units of an ABO type mix that differ from the normal distribution of ABO blood types collected at a routine blood drive. To obtain these units, type-specific recruitment campaigns are necessary, which are labor intensive and therefore more costly to conduct regardless of the region of the country.

The majority of blood centers that seek contractual agreements to import blood are either unable to meet the community's need on a routine basis or experience frequent shortages, often of type-specific units. However, some importing centers may make a deliberate decision to acquire blood units outside the community for financial reasons. In certain metropolitan areas, high labor costs for recruitment and production personnel and/or inefficient blood collection operations drive up the cost of blood. To control costs, these centers enter agreements to obtain a certain percentage of blood units from lower-cost areas. Despite the existence of contractual agreements, importing centers must often purchase blood on an ad hoc basis as well to meet blood needs. One reason for this is that there may be wide fluctuations in demand; another is that the supplying center may not always meet the terms of the agreement. Blood purchased through ad hoc exchanges may be offered at a higher fee and may not include as favorable a blood type mix as blood units obtained through contractual agreements.

Thus, in some areas of the country where the local blood center is not able to supply all of the needs of the area's hospitals and transfusion facilities, the hospitals may establish an in-hospital collection facility, contract with another blood center for blood units, or develop an agreement with one of the national blood inventory management systems.

2.5 Other activities of community blood centers

Traditionally, blood centers carried out a variety of activities that provided services in addition to the blood components. Examples of these other

services include continuing education for physicians, technologists and/or nurses, human leukocyte antigen (HLA) typing, therapeutic apheresis, red cell reference laboratory testing, outpatient transfusions, and medical consultation for transfusion medicine. These services were often provided to hospitals and the medical technical nursing community at little or no extra charge because the activities were subsidized by the income generated from the charges for the blood components. However, as blood centers have attempted to stabilize or reduce their prices to hospitals, it has become necessary for these additional services to become self-supporting financially. In many situations, hospitals have been unwilling to spend money for the services and, as a result, blood centers have reduced or eliminated these activities and are now more narrowly focused on collecting and distributing blood rather than the broader activities they provided in the 1980s.

2.6 The plasma collection system

A method was developed at the beginning of World War II to process large volumes of plasma so that some of the proteins could be isolated, concentrated, and used for medical purposes [34]. This plasma "fractionation" process is the basis for a large industry that provides many medically valuable products generally referred to as plasma "derivatives" [35–38]. There are 22 FDA-licensable plasma derivatives (see Chapter 5). The production of these plasma derivatives is a complex manufacturing process usually involving batches up to 10,000 liters of plasma or plasma from as many as 50,000 donors.

Plasma definitions
The FDA uses two terms for plasma that may serve as the starting material for the manufacture of derivatives: plasma and source plasma. Plasma is "the fluid portion of one unit of human blood intended for intravenous use [39]." This plasma, which is a byproduct of whole blood collected by community blood banks or hospitals, is sold to commercial companies in the plasma fractionation industry, who in turn manufacture the plasma derivatives and sell them in the pharmaceutical market. The blood banks' sale of their plasma to the commercial fractionator (manufacturer) may, but usually does not, involve an agreement to provide some of the manufactured derivatives back to the blood bank.

The amount of plasma obtained from whole blood, estimated to be about two million liters annually [37], is not adequate to meet the needs for raw material to produce plasma derivatives. An additional 12 million liters are collected annually by plasmapheresis [37]. This is called source plasma, which is "the fluid portion of human blood collected by plasmapheresis and intended as the source material for further manufacturing use [39]." Automated instruments are usually used to obtain 650–750 mL of plasma up to twice weekly from healthy adult donors. An individual can donate up to about 100 L of plasma annually in

the United States, if the plasma protein levels and other laboratory tests and physical findings remain normal.

Federally licensed plasma collection and manufacturing organizations

Organizations and facilities may need licenses for either plasma collection or the manufacture of derivatives from plasma, or both, depending on the activities they conduct. As the plasma system developed, it was rather chaotic with blood banks selling their excess recovered plasma, some freestanding centers collecting only plasma, other plasma centers operated by fractionation companies, and some fractionation companies acquiring most of their plasma raw material by contract with plasma centers and blood banks. In the last decade, this system has undergone considerable change, consolidating from ten to four companies operating 443 centers in 40 states [37].

Countries other than the United States have nonrenumerated plasma donor programs; however, few, if any, of these provide all the plasma needs. The United States' system of paid plasma donors produces about 70% of the world plasma supply [37]. Since only about 40% is used domestically, the United States is a major exporter of plasma or finished product derivatives.

Plasma collection activity

Data regarding the plasma derivative industry is proprietary and thus is not readily available. It is estimated that the US plasma and plasma products industry employs over 10,000 people nationwide and produces approximately 14 million liters of plasma annually in the United States [37]. Individuals who donate plasma to support the plasma derivative industry receive between $15 and $20 per donation and it is estimated that donors receive compensation of more than $244 million from plasma collection facilities annually. This is in contrast to whole blood donors, who donate voluntarily and do not receive compensation. Much of the plasma obtained from whole blood collected by blood banks is also used for derivative production. The volume of this plasma can be very roughly estimated as follows: approximately 12 million units of whole blood, suitable for use, are collected annually. If approximately 2 million units are used for fresh frozen plasma and cryoprecipitate, the remaining 10 million units could produce about 2–2.5 million liters of plasma. This combined with the source plasma estimates provide approximately 14 million liters of plasma annually for the production of derivatives.

It is estimated [35] that the worldwide sales of plasma derivatives exceed $4 billion annually, with US firms providing more than 60% of the plasma products or $2.4 billion in domestic and export sales. Of the $2.4 billion in domestic and export sales, $645 million is the estimated export revenue from sales in Europe [35]. It is not known how much of the remaining $1.755 billion sales is domestic and what proportion is from other exports.

2.7 Nongovernmental blood bank organizations

Some organizations such as the American Medical Association, the College of American Pathologists, the American College of Surgeons, or the American Society of Anesthesiologists may from time to time take positions on blood bank and transfusion medicine related issues and maintain blood bank or transfusion medicine committees. The American Society of Hematology includes transfusion medicine in its scientific programs and a section of its journal Blood. Several nongovernmental or professional organizations are devoted exclusively to blood banking and transfusion medicine.

American Association of Blood Banks

The AABB is a professional, nonprofit, scientific, and administrative association for individuals and institutions engaged in the many facets of blood and tissue banking and transfusion and transplantation medicine. AABB member facilities collect virtually all of the nation's blood supply and transfuse more than 80%. Approximately 2,000 institutions (community, regional, and American Red Cross blood centers, hospital blood banks, and hospital transfusion services) and approximately 8,000 individuals are members of the AABB. Members include physicians, scientists, medical technologists, administrators, blood donor recruiters, nurses, and public-spirited citizens. The services and programs of the AABB include inspection and accreditation, standard setting, certification of reference laboratories, operation of a rare donor file, establishment of group purchasing programs, operation of a liability insurance program for blood banks, certification of specialists (technologists) in blood banking, collection of data about the activities of the membership, conduct of regional and teleconference educational programs, provision of professional self-assessment examinations, and conduct of donor recruitment—public education seminars. In addition, the AABB sponsors the world's largest annual meeting where results of new research in blood banking and transfusion medicine are presented; publishes Transfusion, the nation's leading journal reporting scientific, technical, and medical advances in blood banking and transfusion medicine; provides legislative and regulatory assistance to members; develops a wide variety of educational materials for blood bank professionals; and participates in the National Blood Foundation, which provides funds for research in transfusion medicine and blood banking.

Institutional members of the AABB are classified either as a community blood center, a hospital blood bank, or a hospital transfusion service. The community blood center collects blood and distributes it to several hospitals but does not transfuse blood. A hospital blood bank both collects and transfuses blood, and a hospital transfusion service transfuses but does not collect blood. Another way of classifying members of the AABB is the corporate structure of the organization. Of the approximately 2,000

institutional members, 74% are not for profit, 10% are for profit, and a few are government or other types of institutions.

America's Blood Centers

America's Blood Centers (ABC) is an association of independent (non-American Red Cross) not-for-profit community blood centers established in 1962. Efforts to meet the goals of safety, quality, and efficiency in blood services are met through programs such as group purchasing of supplies, services, and liability insurance; efforts to increase volunteer blood donation; effective sharing of blood resources; strengthening of blood center management skills; training programs to ensure compliance with federal regulations; and efforts to ensure fair and balanced resolution of disputes between blood centers and the public they serve. ABC works nationally by helping to shape and influence federal and state regulations, policies, and standards of care that its membership believes are in the best interest of the donors and patients they serve. The association also works to identify and promote needed research and development in blood services; promotes information exchange between members of operational practices; and promotes new programs, policies, and ideas by conducting surveys and meetings of small working groups and by developing workable models. In 2010, the ABC had 77 institutional members with about 600 donor centers in 45 states and Canada that collected about 8 million units of blood, which represented about half of the nation's volunteer donor blood supply.

Plasma Protein Therapeutics Association

The Plasma Protein Therapeutics Association (PPTA) is a trade association that advocates for the world's source plasma collectors and producers of plasma-based therapeutics. Members of PPTA represent nearly every company in the world that collects source plasma for further manufacture. These members operate about 390 plasma collection facilities worldwide and collect about 14 million liters of plasma annually. The role of PPTA is to develop standards and training programs, represent industry to the public and governments, and to provide forums for discussion of new issues or technology.

World Health Organization

The WHO operates a program in Blood Safety and Clinical Technology [2, 17]. The program involves developing guidelines for nationally coordinated blood programs, national blood policies, technical support, a Global Collaboration for Blood Safety program, country-level activities to improve access to safe blood, a Quality Management Project, Guidelines on the Clinical Use of Blood, maintaining the chain of reagents and materials needed for blood operation, laboratory personnel safety, and the safe use of injectables and injection materials. It also sponsors a wide variety of educational symposia worldwide.

Table 2.3 Red Cross and Red Crescent Societies involvement in blood services.

	Total	Americas	Asia Pacific	Europe & Central Asia	Western & Central Africa	Southern Africa	Eastern Africa	Middle East & North Africa
In charge at national level	22	7	6	6	0	0	1	2
Large-scale involvement at branch or district level	26	5	5	4	4	0	2	6
Focus on donor recruitment	112	15	20	32	19	8	9	9
No involvement	8	2	11	1	2	2	1	27
Total	187	35	33	53	24	10	14	18

Source: Global mapping of Red Cross / Red Crescent involvement in blood services. Based on 2008 data kindly provided by Rachel Howden.

Federation of Red Cross and Red Crescent Societies

The Federation, located in Geneva, Switzerland, is the central coordinating organization for all individual country Red Cross or Red Crescent Societies. While most of the Federation's activities involve assistance in times of war or disaster, there is a small blood program office. The latest estimates (Table 2.3) are that approximately 180 National Red Cross or Red Crescent Societies are involved in blood programs. Because of the wide variety of Red Cross involvement and the diverse nature of these Red Cross blood programs, the focus of the blood program at the Federation is on blood donor recruitment. When appropriate, the Federation collaborates with the Global Blood Safety unit at WHO, which is made all the more convenient by their close proximity.

International Society for Blood Transfusion

The International Society for Blood Transfusion (ISBT), founded in 1935, is composed of almost 1,400 medical, scientific, technical, or managerial individuals involved with blood transfusion. Members represent more than 100 countries. The mission of the ISBT is to establish close and mutually beneficial working relationships with international and national professional societies, together with inter-governmental and nongovernmental organizations. By this means, it is possible to disseminate knowledge of how blood transfusion medicine and science may best serve the patient; to create global and regional opportunities for the presentation of research, new developments, and changing concepts in blood transfusion medicine and science and related disciplines; to make provision for the exchange of views and information between members; to promote and to maintain a high level of ethical, medical, and scientific standards in blood transfusion medicine and science throughout the world; and to encourage the development of collaborative programs of good manufacturing, laboratory and user practices in all countries, particularly those with less well-developed blood transfusion services. The

Society publishes Vox Sanguinis, a scientific journal on blood transfusion, transfusion medicine, and blood banking, and a newsletter, Transfusion Today. It also sponsors world and regional congresses, which provide an excellent forum for discussion of not only highly technical subjects and innovations but also issues important to less developed blood transfusion situations.

2.8 Regulation of the blood supply system

United States Federal Regulation

Blood is considered a drug and is regulated by the Food and Drug Administration. The legal basis for this regulation of blood, blood components, and plasma derivatives is provided by two separate but overlapping statutes, one governing "biologics" and one governing "drugs." The biologics law requires that any "virus, therapeutic serum, toxin, anti-toxin, or analogous product" be prepared in a facility holding an FDA license [39]. A separate law, the Pure Food and Drugs Act, covers drugs intended for the "cure, mitigation, or prevention of disease" and thus includes biologics such as blood and blood components or plasma derivatives. Thus, blood banks are subject to the biologics and the drug regulatory process. The federal requirements for blood banks are specified in the Code of Federal Regulations. In addition, the FDA publishes "guidelines" that specify the agency's recommendations of specific policies, procedures, or actions regarding any aspect of the acquisition of blood.

   FDA law requires that all organizations involved in "collection, preparation, processing, or compatibility testing . . . of any blood product" [39] register with the FDA. This registration allows the organization to collect blood and prepare blood components for its own use. If the organization wishes to ship the components across state lines or engage in commerce by selling the products to other organizations, the organization must obtain an FDA license for this purpose. Thus, for practical purposes, most hospitals that collect blood or prepare blood components for their own use are registered, but not licensed, since they do not ship blood in interstate commerce. Most blood centers are licensed, since they supply multiple hospitals, some of which may be in other states. In addition, blood centers may wish to participate in blood resource sharing with blood centers in other states and thus need to be licensed for interstate shipment of blood.

   Federal licensure is intended to ensure that the facility in which the biologic is produced will provide products with high purity and quality. In addition to licensing the facility or establishment, this law requires that each biologic product itself be licensed by the FDA. Thus, to produce a licensed biologic, an organization must have an establishment license describing the facility in which the product is produced and a product license describing the specific product being produced. Over the years, this law has been specifically amended to include the terms blood and blood component or derivative to make it clear that blood is subject to the

biologics regulation. Blood banks and plasma derivative manufacturers are inspected annually by the FDA, and they must submit a report annually to the FDA indicating which products are collected, tested, prepared, and distributed.

When an organization applies for an FDA license to produce blood components or plasma derivatives, it must provide the credentials of the person in charge (responsible head) and of those responsible for determining donor suitability, blood collection, and laboratory processing and testing. In addition, it must provide blueprints and floor plans of the facility; descriptions of all equipment; indications of any other activities occurring in the facility; provisions for housekeeping, pest control, ventilation, lighting, and water systems; other occupants of the building; activity in adjacent buildings; record maintenance systems; validation of all systems; quality control/assurance programs; procedures for receipt and handling of raw materials; source of starting materials; methods and facilities for any chemical purification; inactivation or transfer steps; formulation and final product preparation; computer systems; and other miscellaneous information.

Along with the establishment license, the organization must file a product license application for each product it plans to produce in the facility. For whole blood and components, the product application involves basic information about the manufacturer (organization), facility, product, standard operating procedures, blood donor screening tests, frequency of donation, donor medical history, presence of a physician, phlebotomy supplies, venipuncture technique, collection technique, allowable storage period, storage conditions, disposal of contaminated units, supplies and reagents, label control processes, procedures for reissue of blood, and a brief summary of experience testing 500 samples. For the manufacture of plasma derivatives, the product license application involves the manufacturer's (organization's) name; the establishment name; procedures for determining donor suitability including medical history, examination by physician, laboratory testing, and methods of preparing the venipuncture site and collecting the plasma; methods to prevent circulatory embolism and to ensure return of red cells to the proper donor; minimum intervals between donation and maximum frequency of donation; techniques for immunizing donors; laboratory tests of collected plasma; techniques of preparing source plasma and storing it; methods to ensure proper storage conditions and identification of units; and label control systems and shipping conditions and procedures.

Other required licensure

Blood banks are subject to a number of other requirements or licensure systems in addition to the FDA. The Clinical Laboratories Improvement Act (CLIA) of 1988 established a new section of the Public Health Service Act that requires the Department of Health and Human Services (HHS) to establish certification requirements for any laboratory that performs tests on human specimens and to certify that those laboratories meet the requirements established by HHS. Laboratories participating in the

Medicare and Medicaid programs or engaged in testing in interstate commerce must comply with these CLIA requirements. The law makes it possible for HHS to approve certain accreditation bodies and state licensure bodies. Because blood banks carry out testing on human material that is in interstate commerce, and because they provide services to Medicare and Medicaid patients, they must comply with CLIA. Several states also require that blood banks have a license to operate or provide blood in that state. These licenses usually involve a specific application and inspection.

Voluntary accreditation of blood banks

The AABB operates a voluntary accreditation system. This Inspection and Accreditation (I&A) program, initiated in 1958, involves a biannual inspection by AABB volunteers. The major intent of the I&A program is to increase the safety in obtaining and transfusing human blood and components. The program is also designed to assist directors of blood banks and transfusion services to determine that knowledge, equipment, and the physical plant meet established requirements to detect deficiencies in practice and to provide consultation for their correction. The I&A program can be used to eliminate duplicate inspections by state governments. Many states accept AABB inspection of blood banks and transfusion services to satisfy their licensing requirements. The US Armed Services and Humana, Inc., also maintain an equivalency program with the AABB. The AABB has established a coordinated inspection program with the College of American Pathologists (CAP) in which the CAP and AABB inspections are usually done simultaneously.

CAP Accreditation Program

The CAP, through its Hospital Laboratory Accreditation Program (HLAP), also certifies hospital blood banks but not community blood centers. The HLAP was initiated in 1961 with the primary objective of improving the quality of clinical laboratory services throughout the United States. The HLAP has grown considerably in size, complexity, and effectiveness since its inception, but the primary goal remains that of laboratory improvement through voluntary participation, professional peer review, education, and compliance with established performance standards. The CAP accredits approximately 4,300 laboratories throughout the United States, as well as several foreign countries.

The HLAP examines all aspects of quality control and quality assurance in the laboratory, including test methodologies, reagents, control media, equipment, specimen handling, procedure manuals, test reporting, and internal and external proficiency testing and monitoring, as well as personnel safety and overall management practices that distinguish a quality laboratory.

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3 Recruitment of Blood Donors

In the last 25 years, the requirements for suitability for blood donation have been greatly expanded (see Chapter 4). This combined with more extensive laboratory testing (see Chapter 8) excludes an increasing proportion of the population as potential donors [1–3]. Thus, it is important to understand the motivations of donors and the psychosocial factors that lead to blood donation as a basis for formulating strategies to maintain or increase the number of available donors. To recruit more donors, blood centers have target appeals to specific ethnic groups, streamlined the donor history, begun to collect blood from patients with hemochromatosis, extended upper age limits, and begun to collect blood from 16- and 17-year-olds [4].

3.1 Demographic characteristics of blood donors

Although most Americans will require a blood transfusion at some time in their lives, it is said that less than 5% of the total population or less than 10% of those eligible to donate have ever done so although there are no data to directly support this. Many donors give once or infrequently, and much of the nation's blood supply comes from a small number of dedicated and frequent donors. Blood donors differ from the general population. A variety of demographic characteristics or other factors have been related to the likelihood of an individual being a blood donor. Some of these characteristics include age, marital status, gender, educational experience, occupation, peer pressure, humanitarianism, fear of the unknown, apathy, self-esteem, race, social pressure, altruism, volunteerism, convenience, and community service [5–11]. These characteristics are described in more detail below.

Gender

There is a preponderance of females among first-time donors [5], but with subsequent donations the ratio shifts to a male preponderance of 60–80% [7, 10] with an overall average of 52% of donors males [12]. Deferral has a more pronounced effect on first time compared to repeat blood donors, and since women are more likely to have a reaction during donation and reactions reduce the likelihood of a donor returning, the gender distribution shifts with advancing age to an increasing percentage of men compared to women. The greatest loss of female donors apparently occurs at about the fourth to eighth donation. In general, the larger the number

of lifetime donations the greater the male preponderance [10]. It seems likely that the shift from female to male donors with increasing numbers of donations is a result of deferral of women in the childbearing age who become iron deficient from menstrual blood loss.

Age

Most donors are 30–50 years old, with an average age between 33 and 38 [5, 10, 12]. The age range of donors shifted during the 1980s and 1990s from only about 2–3% of donors over age 60 in the 1970s to 10% of donors over age 60 and 4% over age 65 in the 1990s [10]. This apparent "aging" of the donor population could reflect a shift in the population age in general; however, during the past few years blood bank professionals have recognized that blood donation is safe for older individuals, and donor age limits have therefore been extended to attract older donors (see Chapter 4). During the last few years, recruitment of college and high school students has been emphasized and they now account for about 8% of donations [4]. Younger donors are more likely than repeat donors to experience a reaction [4, 13], since donors who experience a reaction are less likely to return [4, 13–15]. Strategies to minimize reactions in young and first-time donors are being developed (see Chapter 4). Donors most likely to return are those ages 16 and 17 and older than 50, males, blood group O donors, and those without any initial adverse reaction [16].

Race/ethnicity

There are differences in the rate of donation by different ethnic groups. In 1975, whites were 48% more likely to have donated blood during the previous year than nonwhites [5]. By 1989, this figure had increased to 56%. Minority and Latin America born donors are younger and more likely first-time compared with white US born donors, but the annual donation frequency of these minority donors is only slightly lower than white US born donors [17].

Education and socioeconomic characteristics

Blood donors tend to have more education and higher incomes than the general population with incomes as much as 30% higher than those of nondonors [5]. Donors with some college education are the most over-represented group compared with nondonors [3]. Lightman [18] found that 60% of blood donors in Toronto, Canada, had some post-high-school education, compared with 20% for the entire city. Bowman et al. [12] found that 77% of their donors had some post-high-school education, with a range of 60–80% in the different blood centers participating in the study. In a separate study, 69% of all donors and 79% of multi-gallon donors had more than a high-school education [9].

Employment

In Bowman's study [12], 80% of donors were employed full time and another 9% part time. Seven percent were retired, which is consistent with the 10% of donors who were 60 years old or older.

Other social characteristics

It is important to understand donors' behavior that might increase their likelihood of transmitting disease. It appears that donors have fewer sex partners, less frequent sexual experiences, fewer homosexual experiences in males, and are less likely to engage in behavior that puts them at risk for transfusion-transmissible diseases, although about 1.5% report some kind of risk behavior [19]. In one study, 8% of young "potential" donors tested positive for drugs of abuse [20], but since these were not actual donors, it is not clear whether this experience would apply to blood donors.

3.2 Motivation of whole blood donors

Psychosocial theories applicable to blood donation

Piliavin [5] discusses five psychosocial theories that might apply to blood donor motivation. These are (a) Becker's model of commitment, (b) the opponent process theory, (c) the attribution/self-perception framework, (d) the identity theory, and (e) the theory of reasoned action. These can be described briefly as summarized from Piliavin [5].

In Becker's theory of commitment, the action or decision is based on background factors or preconceptions. These factors influence whether the person takes the initial action and then the person is subsequently influenced by the first few experiences. In the opponent process theory, the stronger the negative feelings before the action, the stronger the positive feelings after successfully completing the action. Thus, despite initial fears or negative feelings, a good experience with donation could lead to a strongly positive attitude about continued donation. This theory attempts to account for the continuation of activities that were initially associated with negative feelings. The attribution theory postulates that if an individual believes that there is an external reason for the action, the action is attributed to that external force. In the identity theory, the sense of self is developed from the variety of social roles in which the individual engages. In the theory of reasoned action, the critical factor leading to an act is the development of an intention to carry out the act.

Integrated model

Piliavin [5] believes that all five of the theories outlined above have some relevance to blood donation. The identity theory is thought to be "overarching" and that the "sense of self as donor is clearly the central factor among the personal determinants of donation." Becker's model applies to making the commitment to donate, the opponent-process and attribution theories relate to the development of a sense of the individual as a blood donor, and the theory of reasoned action is involved in translating the person's idea of himself or herself as a blood donor into action to donate. In integrating these theories, Piliavin [5] believes that the decision to donate is based on "childhood experience factors" negatively influenced by "pain and inconvenience" and positively influenced by "social pressures."

Theory of planned behavior

This model is based on attitude, subjective norm, and perceived behavioral control [21]. These influence the intention to carry out behaviors. While this model has a different name than those of Piliavin [5], theory of planned behavior (TPB) really involves attitudes about the behavior, social pressure about the behavior, and control over performing the behavior. Thus, it is not surprising that several studies (reviewed in 21) indicate that TPB predicts a substantial portion of blood donation intention and behavior [21].

Giving and not giving

The reasons for donating are summarized generally [5] as (a) extrinsic rewards and incentives, (b) intrinsic rewards and incentives, (c) perceived community needs, (d) perceived community support, (e) social pressure, and (f) addiction to donation. The reasons for not donating include (a) medical ineligibility, (b) fear, (c) reactions and deferral (poor experiences), and (d) inconvenience and time requirements. Oswalt [7], in reviewing 60 English-language reports regarding motivation for blood donation, concluded that the following factors were motivations to donate: (a) altruism and humanitarianism, (b) personal or family credit, (c) social pressure, (d) replacement, and (e) reward. Reasons for not donating included (a) fear, (b) medical excuses, (c) reactions, (d) apathy, and (e) inconvenience. Rados [22] also found that fear, inconvenience, and never being asked were the most common reasons given for not donating. In general, the issues described above have seemed to appear rather consistently in these and other studies [23] of donor motivation or nondonation. Because they have been consistent over time, most recruitment strategies attempt to take these factors into consideration. Most blood donors have a rationale way of thinking about blood donation but some think and make decisions about blood donation based on emotional, personal, or stereotype manner and are interested in continuing to donate blood but the convenience of donation is a stronger factor than helping others [24]. In general, donors give blood out of altruism and in response to a general appeal or a specific request.

3.3 The donation experience and factors influencing continued donation

About 28% of blood is collected from first-time donors, about half of whom return within the first year [25]. Those who return tend to be white, United States born, and college educated [25]. Experience with the first donation has a major effect on their willingness to return for subsequent donations [5, 14, 16]. Although the first blood donation is anxiety producing, it is usually accompanied by good interactions with the donor staff and good feelings about the donation and oneself [26]. Thus, most donors realize that they are reasonably able to give and plan to do so again.

With continued donation, the experience becomes easier and the reasons for continuing to donate become more "internal" [5].

About 80% of donors are repeat donors, although this percentage is decreasing [16, 27], and repeat donors tend to be 16 and 17 or older than 50, male, Rh negative, type O, without a reaction during donation and have a college degree [16, 28]. A shorter interval between the first two donations also predicts more continued donations [28]. Over time a "blood donor role" develops in repeat donors, and this strengthens self-commitment to blood donation, including "friendships contingent on donating, a self-description as a regular donor, an increase in the ranking of the blood donor role, greater expectations from others, and even more donations" [5].

Donors who are deferred are less likely to return to donate after the reason for the temporary deferral has passed [5, 16]. This is not surprising since deferral breaks the good feelings that might have developed about donation and makes future donation more difficult. Experiencing a reaction also reduces the likelihood of a donor returning [5, 14, 15]. This is because the donor begins to see himself or herself as someone who has trouble donating, and the reaction experience modifies any previous positive feelings about donation. Surprisingly, most multi-gallon donors report that they do not receive recognition for their donation, and the knowledge that a friend or relative was a blood donor did not make them more likely to donate [10]. These observations are consistent with the general view that the initial donation is motivated primarily by external factors and continued donation primarily by internal factors [29].

Social influences on blood donation

Because blood donation is not done in private, it is by definition a "social" act [5]. It is not surprising that social factors and issues have a strong influence on blood donation. Social norms of the community affect donation behavior. For example, general publicity about blood donation in the community creates the perception that blood donation is an active part of the community, thus setting the stage for positive decisions by individuals to donate. This, along with "intrinsic" motivational appeals, builds a community norm for blood donation.

Volunteerism plays a major role in blood donation. A sense of social connection is gained, a feeling of helping others, and a history of volunteerism in the family or during their school years characterize blood donors [11].

Family history of donation or blood use

Blood donors are more likely to have had family members who were blood donors [5]. It is not clear whether blood use by a relative or close friend influences the likelihood that one will be a blood donor [7].

The donation situation

Blood is collected in both fixed and mobile sites. Fixed sites are facilities that are permanently outfitted to serve as a blood donation center. These

sites are usually within the blood center or hospital but may be in freestanding locations such as office buildings or shopping malls. The proportion of blood that is collected in fixed sites is not known. A very large portion of the US blood supply is collected in mobile sites. A mobile site is a location that usually serves a purpose other than blood donation. At the mobile site, or "bloodmobile," all of the equipment and supplies necessary for blood collection are portable and are brought in for a few hours or days for the blood collection activity. These mobile sites are in many other kinds of settings such as offices, high schools, social clubs, churches, colleges, manufacturing companies, public buildings, or shopping malls. Donors at mobile sites are more likely to be first-time donors, giving under social pressure, and thus with less internal motivation to donate, and they are more likely to experience a reaction or less-than-optimal experience [5]. However, the influence that these settings have on the likelihood that an individual will donate or will have a good experience and be willing to donate again is not well understood. There are differences between those who donate at fixed sites and those who donate at mobile locations [5]. Donors at fixed sites report more internal motivation, whereas those at mobile sites report more external motivation. This would be consistent with the structure of mobile sites, which are usually arranged around a blood "drive" of some sort involving a community group or a particular need, thus providing the "external" motivation. At fixed sites, the donor is usually called by blood center staff and the donation scheduled as part of the general ongoing blood collection activity, but there is no relation to a particular community or social group or patient.

Organizational influences

There are different types of organizations involved in collecting blood and providing the nation's blood supply (see Chapter 2) and these may influence people's willingness to donate. Such preferences could be due to the nature of the organization or the individual donor's motivation. One major difference among organizations is hospital-based donor programs versus community-based freestanding blood centers. Because the community-based blood center serves multiple hospitals, the organization (and, by extension, a donation made to it) carries an image of service to the general medical community along with a feeling of community pride and allegiance. The nature of the community organization may also influence the individual's willingness to donate. About half of the blood collection in community centers is carried out by the American Red Cross. The other half of blood collection is performed by freestanding organizations whose mission is the provision of blood services. In contrast, a hospital-based program is associated with a specific medical center and can take advantage of the image of that center and its physicians, programs, and patients. Although there are no definitive studies, it seems likely that these factors influence the types of donors and their motivation for donating to different organizations. The differences between organizations involved may also affect the setting in which the blood is collected and thus may indirectly influence the donors or their motivation. For instance,

hospital-based donor programs are more likely to use fixed sites for blood collection. While community-based blood centers use some fixed sites, they use mobile sites more extensively than hospital programs.

Role of incentives

A variety of incentives, ranging from small trinkets such as key chains, coffee mugs, or T-shirts to tickets to events to cash, have been offered to donors in hopes of motivating them to continue to donate. In almost every study worldwide, paying donors results in donors with a higher likelihood of transmitting disease [30, 31]. Thus, organizations such as the American Association of Blood Banks, the American Red Cross, the International Society for Blood Transfusion, the World Health Organization, and most countries that have a national blood policy stipulate that blood for transfusion be obtained from volunteer donors. The definition of volunteerism in blood donation is whether the incentive is transferable, refundable, or redeemable or whether a market for it exists [32]. If none of these applies, it is presumed that the incentive could not be converted into cash.

In some very specific situations, it is possible to pay donors without increasing the risks of transmissible disease [33, 34], although this is not recommended. Blood testing for cholesterol or prostate specific antigen or blood "credits" may be an incentive for many donors [35, 36], and incentives help to attract first-time and younger donors [31]. Some blood banks have used a blood "credit" system in which nondonors are charged a higher fee for the blood as an incentive to replace blood used. This practice is no longer used in the United States, but in many countries with an inadequate blood supply, versions of this practice are used [37].

3.4 Whole blood donor recruitment strategies

It is generally believed that the most effective way to get someone to donate blood is to ask directly and personally [5, 8, 38]. For instance, in one study comparing two recruitment techniques, the direct "foot in the door" approach was found to be superior to a general request to participate in a donor program [39]. When people were asked their reasons for not donating, the most common response was "no one ever asked" [8]. The wide variety of settings in which blood is collected may also affect an individual's willingness to donate. Different motivations may apply to donation in different settings, or the setting may influence an individual's willingness to donate. However, for either the fixed or mobile site, substantial efforts are made to specifically ask the individual to donate and, if possible, schedule the donation. This request may be made by paid staff of the donor organization or by volunteers. It is not clear whether either type of individual is more effective. Many blood centers with large donor programs have implemented computerized calling systems that integrate the donor files with the donor's history along with automated dialing systems to maximize the efficiency of the telephoning process.

Usually blood donors are asked to give to the general community blood supply. Sometimes donors are asked to donate blood for one or more specific patients. Newman et al. [40] found that these patient-related blood drives were easier to organize, produced more blood, and left the donors and staff with a stronger sense of satisfaction because of the more personal nature of the experience.

Very little structured social science research has been directed to the issue of minority involvement in the blood donation process. Some success in increasing blood donation by minorities has been achieved by involving more minority staff in the recruitment and blood donation process (Wingard M, quoted in reference 2]. Compared with Hispanic nondonors, Hispanic donors were found to be better educated, to be more likely to speak English, to have higher job status, and to be more likely to have parents who were donors [41]. The study concluded that there is a need for improved education for Hispanic donors about the safety of blood donation. With the general aging of the population and the extending of age limits for blood donation, donation has been found to be safe for older persons. However, there are no unique recruitment strategies targeted to older donors. As more blood is collected at colleges and high schools, the materials and publicity are designed to appeal to that age group and so could be considered to be targeted to this population segment.

Another issue in donor recruitment is whether to devote more effort to recruitment of new donors or to maintaining existing donors. New donors add to the overall files and replace donors inevitably lost due to attrition or disqualification. Thus, it is essential to replenish the donor pool. However, once people are in the donation habit, strategies to encourage them to continue result in the collection of substantial amounts of blood for less effort than is required to recruit new donors. Therefore, the dilemma is not in choosing only one of these strategies, but in balancing the effort between them to maintain an adequate donor file and also to produce new donors with a reasonable amount of effort.

3.5 Apheresis donor recruitment

During early phases of the development of cytapheresis, donors were usually friends or relatives of the patients or else they were staff members of the hospital or blood bank. For instance, in one of the first platelet donor programs (established at M.D. Anderson Hospital in 1963), there was no organized donor recruitment program [42]. Instead, the donors themselves, as well as blood bank staff, served as informal recruiters. As it became clear that cytapheresis would become a more widely used method of producing blood components and the procedures began to take a place in the more routine operation of blood centers and hospitals, attention was directed to more formal and structured donor recruitment programs. This raised considerations in addition to those for whole blood donors. The cytapheresis procedure is longer and thus requires more of a time commitment. In addition, the side effects, the nature of adverse reactions

to donation and the donor medical assessment might be different from whole blood donation. Thus, the types of people to be approached about donation, the information they would be given, and the strategy to be used to obtain the best decision from the donor and potentially the highest acceptance rate became topics of great interest.

A key step in the development of cytapheresis donor programs was a conference that was held to address the scientific, legal, and ethical issues [43]. Issues such as the cost-effectiveness of platelet transfusion, individual rights, informed consent, donor decision-making mechanisms, and personal autonomy were discussed in the context of plateletpheresis donation. The results of this conference formed a sound basis for the development of cytapheresis donor programs. Because of the additional burden of cytapheresis donation, frequent whole blood donors were selected as possible cytapheresis donors. The provision of informational materials was often sufficient to attract them into cytapheresis donation [44]. Platelet donation was not only an altruistic act of giving, but it also filled some personal needs of the individual.

Apheresis now is the major platelet production method (see Chapter 2) occurring thousands of times each day. Recruitment continues to focus on successful whole blood donors who are provided information about apheresis donation. Some blood centers position the apheresis collection area within site of whole blood donors as a recruitment technique. Since apheresis donation can be done more frequently than whole blood, this is another incentive for some donors.

3.6 Bone marrow donors

With increasing use of bone marrow transplantation to treat a wider variety of diseases and with the success of the treatment improving, the lack of a suitable family donor for most patients became a major limitation in the availability of this treatment. The first successful transplant using marrow donated by a volunteer not related to the patient [45] opened the urgent need for large numbers of HLA-typed individuals who would be willing to donate marrow. A remarkable story unfolded, resulting in the establishment of the National Marrow Donor Program [46].

Initially it was believed that it might be improper or even unethical to ask volunteers unrelated to the patient to donate marrow. The risks of donation, the discomfort, the nature of the patient's preparation for the transplant, and lethal consequences of withdrawal by the donor all contributed to an atmosphere in which this kind of donation seemed unlikely. However, the possibility was pursued in a small number of centers [46]. One of the major first steps was a conference sponsored by the University of Minnesota at which the legal, ethical, social, financial, and practical issues in unrelated volunteer marrow donation were discussed [47]. The involvement of two community blood centers added strength at this early stage because they were separate from the transplant centers and also because of the involvement of representatives of the general

community as part of their governing boards. As these organizations began to consider establishing a marrow donor program, the results of the conference strengthened the belief that there were proper ways to inform people of the opportunity and the consequences of donation and to provide the opportunity to become a donor if desired [47]. The initial ethical principles involved respect for life, promotion of good, prevention of harm, justice, fairness, truth-telling, and individual freedom [47].

Because of the extensive commitment required of donors, it was decided to approach multi-gallon blood donors and apheresis donors [48, 49]. This had the added benefit that most were already apheresis donors who had been HLA-typed, and this avoided the cost of additional HLA typing. The "recruitment" involved providing an extensive description of marrow transplantation, the situations in which it was used, the results of transplantation including actual survival statistics, the marrow donation process, and the steps that would lead up to marrow donation. The recruitment process drew heavily on the considerable experience of sociological studies of families making the decision to donate an organ either to a relative or for cadaver transplantation [50, 51]. The informed consent process was given very heavy weight in the recruitment process [48]. Remarkably, about three-fourths of the donors who were provided an extensive description of the marrow donor program elected to participate [48, 49]. Important factors in the donor's decision whether or not to participate in the program were religion, experience with the medical system, and the spouse's attitude regarding marrow donation [49].

Donor recruitment efforts were expanded to the general public. Although there was concern that people who had never donated blood would not be sufficiently well informed or willing to make the necessary commitment, general community appeals for donors resulted in the recruitment of donors who became as committed to the program as the cytapheresis donors [45]. The national marrow donor file has now grown to more than 8 million volunteers in the United States and many more worldwide. The extensive experience with marrow donation establishes the effectiveness of the recruitment process and the lifesaving impact of the therapy on patients.

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4 Blood Donor Medical Assessment and Blood Collection

Because blood is considered to be a drug and is regulated under US Food and Drug Administration (FDA) law, most aspects of the selection of potential donors and the collection of blood are carried out under requirements established by the FDA. This chapter attempts to provide concepts and rationale for blood donor assessment and blood collection but does not refer to every specific FDA requirement. However, it should be understood that all of these activities must conform to FDA requirements, which can be found in the Code of Federal Regulations and various FDA guidelines. For blood banks that desire accreditation by the American Association of Blood Banks, the standards of that organization must also be followed. The Technical Manual of the American Association of Blood Banks [1] is an excellent reference that provides details for much of the content of this chapter.

4.1 Medical assessment of whole blood donors

The approach to the selection of blood donors is designed around two themes: to ensure the safety of the donor and to obtain a high-quality blood component that is as safe as possible for the recipient. In general, 10–15% of presenting donors are either deferred or provide an unsatisfactory unit of blood [2]. This is due to short-term deferrals (e.g., hemoglobin), long-term deferral (malaria), permanent deferral (hepatitis), disease marker reactive donations, or an unsatisfactory collection process [2] (Table 4.1). An additional 1.2% of units are rejected due to positive transmissible disease tests [3]. The loss of this many potential donors has a huge impact on the blood supply [4].

Registration

A major factor that influences whether blood donors will make subsequent donations is the experience donors have at each donation. Thus, it is important that the blood collection staff provide a warm, friendly, professional, and efficient environment in which the medical assessment and blood donation can take place.

Table 4.1 Strategies for safe blood.

Using only volunteer blood donors
Questioning donors about their general health before their donation is scheduled
Obtaining a medical history before donation
Carrying out a physical examination before donation
Carrying out laboratory testing of donated blood
Checking the donor's identity against a registry of previously deferred donors
Providing a method by which after donation the donor can confidentially designate the unit as unsuitable for transfusion

When the donor initially presents at the donation site, identifying information is obtained for the permanent record. This includes name, address, telephone number, birth date, social security number (if allowed in that state), and previous donation history including any names under which previous donations might have been made. In order to prevent iron depletion, individuals may donate no more often than every 56 days. At the time of registration, the prospective donor is given information about blood donation, transmissible disease testing, and factors or behavior that would preclude blood donation. Information may also describe the agencies that are notified in the event of a positive test result for a transmissible disease. While obtaining the medical history, a staff member asks the donor questions about these factors and describes the risk behavior for human immunodeficiency virus (HIV) infection. It is required that the identity of each donor be checked against a registry of individuals known to be unacceptable as blood donors [5]. It is assumed that people have no reason to donate blood under false names, and so this questioning is done primarily to identify women whose name may have changed due to marriage. Some blood banks do this "donor deferral registry" check at the time of registration to avoid collecting blood from anyone on the registry, and other blood banks carry out this check at the blood center later before the unit of collected blood is made available for distribution. Although this process is required by the FDA and is widely used throughout the United States, there has never been a thorough study to establish its value and some registries in close proximity do not share the information.

For many years people were deferred from donating blood after 65 years of age. However, as the population has aged, with many more people living active, healthy lives well beyond their 65th year, the arbitrary use of an age limit for blood donation has been eliminated. Elderly donors have more medical conditions and medications than younger donors but do not experience more adverse reactions to donation [6,7]. Elderly donors have slightly decreased iron stores [8], but these donors can safely contribute to the nation's blood supply. Schmidt [9] points out that "blood donation by the healthy adult is remarkably safe and data show that this is true well into old age." Thus, most blood centers do not have a specific upper age limit for blood donors but evaluate donors on an individual basis. The lower age limit for blood donation is usually 17 years, but some states have passed laws that allow donation at the age of 16 even though these are below the

age of 18 at which individuals can take legal responsibility for their actions. These special laws dealing with blood donation are intended to enable high school students to donate without obtaining parental consent. However, younger donors may have a higher risk profile [10] and so the importance of an accurate history must be especially stressed with these donors.

Medical history

The medical history is an extremely important part of the selection of donors because it can reveal reasons why donation might not be wise for the donor or reasons why the donor's blood might constitute an increased risk for the patient. The interview usually takes about 10 minutes. In addition to specific questions (Table 4.2), the interviewer attempts to establish some rapport with the potential donor and to make an assessment of the donor's general condition. This is important to establish that the donor is in good general health, is not under the influence of drugs, and is able to give informed consent for the donation. The interview must be conducted in a setting that provides privacy for the donor. While complete visual privacy is not always possible, visual distractions should be minimized and the donor's answers must not be audible to others. The interview consists of some questions that the donor answers in writing and some verbal responses to questions asked by the interviewer. Several different ways of seeking information from the donor have been used. These include different kinds of brochures, videos, and direct questioning. In studies comparing these approaches, direct questioning elicits the most accurate information [11,12]. Computer-assisted donor screening seems to be acceptable to donors and may decrease errors [13]. The nature of these sex-related questions is very specific, and the act of directly questioning the donor about these behaviors has certainly changed the interaction and relationship between the donor and the blood bank [14].

An interagency task force developed a standard donor history questionnaire that is approved by the FDA and now used by most blood banks. The questions designed to protect the safety of the donor include those regarding age and medications and whether the donor is under the care of a physician or has a history of cardiovascular or lung disease, seizures, present or recent pregnancy, recent donation of blood or plasma, recent major illness or surgery, unexplained weight loss, or unusual bleeding. Questions designed to protect the safety of the recipient include those related to the donor's general health; the presence of a bleeding disorder; receipt of growth hormone; exposure to or history of jaundice, liver disease, hepatitis, AIDS (or symptoms of AIDS), Chagas' disease, or babesiosis; the injection of drugs; receipt of coagulation factor concentrates, blood transfusion, a tattoo, acupuncture, ear piercing, or an organ or tissue transplant; travel to areas endemic for malaria; recent immunizations; contact with persons with hepatitis or other transmissible diseases; ingestion of medications, especially aspirin; presence of a major illness or surgery; or previous notice of a positive test for a transmissible disease. Donors with a history of hepatitis before age 11 are not deferred because that hepatitis was likely type A, which does not have a carrier state.

Table 4.2 Complete list of medical history questions for blood donors.

Are you
Feeling healthy today?
Currently taking an antibiotic?
Currently taking any other medication for an infection?

Please read the medication deferral list
Are you now taking or have you ever taken any medications on the Medication Deferral List?
Have you read the educational materials?

In the past 48 hours, have you
Have you taken aspirin or anything that has aspirin in it?

In the past 6 weeks, have you
Female donors: Have you been pregnant or are you pregnant now? (Males: check "I am male")

In the past 8 weeks, have you
Donated blood, platelets or plasma?
Had any vaccinations or other shots?
Had contact with someone who had a smallpox vaccination?

In the past 16 weeks, have you
Have you donated a double unit of red cells using an apheresis machine?

In the past 12 months, have you
Had a blood transfusion?
Had a transplant such as organ, tissue or bone marrow?
Had a graft such as bone or skin?
Come into contact with someone else's blood?
Had an accidental needle-stick?
Had sexual contact with anyone who has HIV/AIDS or has had a positive test for the HIV/AIDS virus?
Had sexual contact with a prostitute or anyone else who takes money or drugs or other payment for sex?
Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything not prescribed by their doctor?
Had sexual contact with anyone who has hemophilia or has used clotting factor concentrates?
Female donors: Had sexual contact with a male who has ever had sexual contact with another male? (Males: check "I am male")
Had sexual contact with a person who has hepatitis?
Lived with a person who has hepatitis?
Had a tattoo?
Had ear or body piercing?
Had or been treated for syphilis or gonorrhea?
Been in juvenile detention, lockup, jail, or prison for more than 72 hours?

In the past 3 years, have you
Been outside the United States or Canada?

From 1980 through 1996
Did you spend time that adds up to three (3) months or more in the United Kingdom? (Review list of countries in the UK.)
Were you a member of the US military, a civilian military employee, or a dependent of a member of the US military?

From 1980 to the present, did you
Spend time that adds up to five (5) years or more in Europe? (Review list of countries in Europe.)
Receive a blood transfusion in the United Kingdom or France? (Review list of countries in the UK.)

From 1977 to the present, have you
Received money, drugs, or other payment for sex?
Male donors: had sexual contact with another male, even once? (Females: check "I am female")

Table 4.2 (*Continued*).

Have you ever
Had a positive test for the HIV/AIDS virus?
Used needles to take drugs, steroids, or anything not prescribed by your doctor?
Used clotting factor concentrates?
Had hepatitis?
Had malaria?
Had Chagas' disease?
Had babesiosis?
Received a dura mater graft?
Had any type of cancer, including leukemia?
Had any problems with your heart or lungs?
Had a bleeding condition or a blood disease?
Had sexual contact with anyone who was born or lived in Africa?
Been in Africa?
Had any relatives who had Creutzfeldt–Jakob disease?

Source: Food and Drug Administration. Guidance for industry: implementation of acceptable full-length donor history questionnaire and accompanying materials for use in screening donors of blood and blood components. Rockville, MD: CBER Office of Communication, Training, and Manufacturers Assistance (October 2006).

Many believe that the question about hepatitis should be eliminated, but the FDA has not done so.

Individuals who have spent more than three months in the United Kingdom are deferred because of possible exposure to the causative agent of variant Creutzfeld–Jakob disease (CJD) [15]. FDA recommended travel referrals to reduce the potential transmission of variant CJD projected donor losses ranging from 1% to 13%, but experience has shown a rate of approximately 1.6% [16].

Potential donors who have resided in or traveled to malaria endemic areas are deferred for 12 months. However, most transfusion-transmitted malaria is associated with lengthy residence in malaria endemic areas rather than routine or short-term travel. Travel to Africa results in a malaria infection rate 1,000 times that of travel to malaria endemic areas of Mexico, yet Mexico accounts for more than ten times as many deferred donors. Shortening the deferral period from 12 to 3 months for travelers to Mexico increases the risk of a contaminated unit by only one unit per 57 years in the United States yet an annual gain of more than 56,000 donations [17].

Several questions related to AIDS risk behavior include whether the potential donor has had sex with anyone with AIDS, given or received money or drugs for sex, had sex with another male (for males), or had sex with a male who has had sex with another male (for females).

One area of inconsistency is that males who have had sex with a male at any time since 1977 (when the HIV virus is thought to have entered humans) are deferred while males who have had sex with a female prostitute or used IV drugs are deferred for only one year. In one study of 21,168 male donors, 24% reported having male-to-male sex and most of

this was since 1977. In those donors who reported male-to-male sex in the last five years, the prevalence of reactive screening test results was higher. In addition, the prevalence of other unreported risks was higher in donors who reported male-to-male sex at any time. Thus, this study did not provide evidence to support changing the current policy of donor deferral [18].

Previous transfusion recipients could harbor unknown infectious agents and perpetrate the cycle of transmissible disease and so deferral of previous transfusion recipients has been considered. In one very large study, a previous transfusion history was found in 4.2% of donors. However, transfused and nontransfused donors had a similar incidence of positive viral screening tests and other deferrable risks [19].

The medical history is an extremely effective part of ensuring the safety of the blood supply. For instance, the implementation of questions about behavior that would put potential donors at risk for HIV infection decreased the HIV infectivity of blood in the San Francisco Bay area by 90% even before the use of the HIV screening test [20]. Blood donors are less likely than the general public to have engaged in risk behaviors, although 1.5% have done so [21]. One concern has been that people who have engaged in high-risk behavior might seek to donate blood to obtain a test for HIV. In some situations this seems to be true. Of 30 HIV-positive blood donors in Paris, 47% had known risk behaviors and 50% admitted to having donated to obtain a test for HIV [22]. In a larger study of HIV-seropositive blood donors, the reasons for donation in spite of having participated in behavior that placed them at risk of HIV infection were failure to read carefully or comprehend the deferral information, group pressure, a desire to be tested, and belief that the testing would identify any infected blood [23].

Occasionally, situations arise in which the donor's physician believes that donation would be safe, but the blood bank does not accept the donor. For instance, because the genesis of malignant disease is not known, donors with a history of cancer (other than minor skin cancer or carcinoma in situ of the cervix) are usually deferred, although it is not expected that transfusion of blood from these donors would transmit cancer. Some medications may make the individual unsuitable as a blood donor because of the condition requiring the medication, while other medications may be potentially harmful to the recipient. Many other conditions must be evaluated individually by the blood bank physician, whose assessment of conformance with FDA regulations—which consider blood a pharmaceutical—may not always coincide with another physician's view of the health of the potential donor.

Hemochromatosis patients as blood donors

Hereditary hemochromatosis is due to alterations of the HFE gene. The use of phlebotomy to reduce iron stores and prevent progression of the disease continues to be the therapy of choice. Blood obtained by therapeutic phlebotomy of hemochromatosis patients has not been acceptable for transfusion when the pathogenesis of the disease was not understood.

Approximately two-thirds of hemochromatosis patients are probably eligible as blood donors, and it is estimated that about 65% of the units drawn during iron depletion therapy would be suitable for transfusion providing 200,000 to 3 million units [24] of blood annually in the United States. The risk of transfusion-transmitted infections is not greater from hemochromatosis than regular blood donors [25]. Canada has accepted patients with hemochromatosis as donors, and potential use of blood from hemochromatosis patients has been proposed for the United States [26, 27].

Physical examination of the blood donor

The physical examination of a potential donor includes determination of the potential donor's temperature, which can be falsely increased or decreased by hot or cold beverages or gum chewing [28], pulse, blood pressure, weight, and hemoglobin. Each of these has FDA-mandated limits. In addition, the donor's general appearance and behavior are assessed for any signs of illness or the influence of drugs or alcohol. The skin at the venipuncture site is examined for signs of intravenous drug abuse, lesions suggestive of underlying disease, and local lesions that might make it difficult to cleanse the skin and thus lead to contamination of the blood unit during venipuncture.

There are weight requirements for donors because it is necessary to balance the amount of blood collected in relation to the donor's estimated blood volume and also the amount of blood in relation to the volume of anticoagulant in the collection container. To integrate the volumes of blood collected, which can range from about 505 to 575 mL, with the weight ranges of donors, an arbitrary lower weight limit of 110 lb has been established. There is no upper weight limit, although extremely obese potential donors may have other health problems or inadequate venous access-precluding donation. The pulse should be regular and between 50 and 100 per minute, although potential donors who have a slower pulse due to involvement in an active exercise program may donate with approval of the transfusion medicine physician.

The hemoglobin may be tested by a screening method in which a drop of blood is placed in a copper sulfate solution of a known specific gravity so that the falling of the blood drop within the solution indicates an adequate hemoglobin content. A microhematocrit is now often used, but in less well-developed countries, a hemoglobin color scale can be used [29]. The blood drop should be obtained from the finger. Blood from an earlobe puncture can have a falsely elevated hemoglobin [30, 31]. Noninvasive methods for hemoglobin are now available but have not yet become widely used. If the hemoglobin screening test indicates a hemoglobin level below than required for donation, usually a microhematocrit is performed. The minimum acceptable hemoglobin is 12.5 g/dL for men and women. Factors that affect the rate of deferrals due to hematocrit are the proportion of female donors, smokers, African Americans in the donor population, altitude of the donation location, and source of blood sample (finger versus ear lobe). Because the hemoglobin fluctuates with temperature, being lower in hot weather, there may be an increase in

deferrals due to low hemoglobin during the summer months [32]. The use of the same hemoglobin criterion for men and women is inconsistent. The FDA minimum of 12.5 grams/dL is within the normal range for Caucasian women [11.6 to 15.7 grams/dL] but below the normal range for Caucasian men (13.3 to 17.2 grams/dL). Most hemoglobin deferrals occur in women and a large percentage of women who have hematocrits of 36% or 37% that are within their normal limits [33% to 45%]. Thus, many women with essentially normal hemoglobins are being deferred. In contrast, a hemoglobin of 12.5 g/dL may indicate mild anemia in a man. Changing the hemoglobin requirement from 12.5 to 12.0 g/dL for women could increase the US blood supply by approximately 5% [33, 34].

Special blood donations

There are several situations involving blood donation in which the blood may be collected by the donor center but will not be used as part of the community's general blood supply. Examples of these include autologous donation, directed donation, patient-specific donation, and therapeutic bleeding (see Chapter 6). In some of these situations, the FDA requirements for blood donation may not apply.

4.2 Collection of whole blood

The time during blood collection provides another opportunity for the blood center staff to interact with the donor to reinforce the professionalism and set the stage for the donor's willingness to return for subsequent donations. In a way this becomes the first step in the recruitment of the donor for the next donation (see Chapter 3).

Labeling

The first step in the collection process is labeling of all containers, tubes, and related materials. This is an extremely important step because it relates all tubes, specimens, documents, and components to the identity of the donor. Virtually all blood banks use bar-coded labels, and much of the subsequent tracking of specimens, test results, and individual components is done by computers using these labels. Computer systems are generally used to accumulate all data relevant to the individual donation to determine whether the components are suitable for transfusion and can be released into usable inventory. Thus, there are detailed and specific steps in the process at the donor bedside to ensure the accuracy of all materials.

Blood containers

Blood must be collected into FDA-licensed containers, each of which is sterile and can be used only once. The containers are made of plasticized material that is biocompatible with blood cells and allows diffusion of gases to provide optimal cell preservation (see Chapter 5). These blood containers are combinations of multiple bags connected by tubing so that components can be transferred between bags without being exposed to air.

This is referred to as a "closed system." This system of separation of the whole blood into its components in a closed system thus minimizes the chance of bacterial contamination while making it possible to store each component under the conditions and length of time that are optimum for that component. Since some bacteria can enter the blood bag from skin at the venipunture, most blood collection sites now contain a small pouch for diversion of the first few mL of blood. Diversion of the first few mL of blood collected can reduce the bacterial contamination rate from 46–71% [35, 36]. In 2006, 37% of whole blood and 50% of apheresis products were collected using a diversion pouch [3].

Anticoagulant preservative solutions

Several anticoagulant preservative solutions are available. The anticoagulants are various formulas of citrate solutions. The blood may be stored in these solutions and used for transfusion, or most of the supernatant may be removed and the cells stored in other "additive" solutions. The composition and effects of these anticoagulant and preservative solutions are discussed more completely in Chapter 5.

Selection of the vein and preparation of the venipuncture site

Blood is drawn from a vein in the antecubital fossa. The vein selected should be large enough to accommodate a 16-gauge needle. Careful selection of the vein makes the venipuncture quick and easy, thus providing good blood flow and a quality component but also minimizing the discomfort to the donor and making the donation experience as pleasant as possible. The choice of the vein will also minimize the likelihood of inadvertently damaging a nerve or puncturing an artery (see section on Adverse Reactions to Donation). A blood pressure cuff is usually used to impede venous return and distend the vein.

To minimize the chance of bacterial contamination, the blood must be drawn from an area free of skin lesions, and the phlebotomy site must be properly cleansed (Table 4.3). It is not possible to sterilize the skin, but steps are taken to greatly reduce the level of skin flora. This essentially involves scrubbing with a soap solution, followed by tincture of iodine or iodophor complex solution [37, 38]. The selection of the venipuncture site and its sterilization are very important steps, since bacterial contamination of blood can be a serious, even fatal complication of transfusion (see Chapter 14).

Venipuncture

Most blood collection equipment uses 16-gauge needles, and the entire set is closed and connected so that the needle is integral. The venipuncture is done with a needle that can be used only once so as to avoid contamination. Most phlebotomists use a two-step process in which the needle first penetrates the skin, then after a brief pause the needle is inserted into the vein. The pause is so brief that it may not be noticeable to

Table 4.3 General procedure: donor arm preparation for blood collection.

- Apply tourniquet or blood pressure cuff. Identify venipuncture site; then release tourniquet or cuff.
- Scrub area at least 4 cm in all directions from intended venipuncture site for a minimum of 30 seconds with 0.75% or 10% disposable povidone-iodine scrub solution stick.
- Apply preparation solution of 10% povidone-iodine starting at intended venipuncture site and moving outward in a concentric spiral. Let stand for 30 seconds or as directed by manufacturer.
- Cover area with sterile gauze until the time of venipuncture. After the skin has been prepared, it must not be touched again. Do not repalpate the vein at the intended venipuncture site.

Sources: Goldman M, Roy G, Fréchette M, et al. Evaluation of donor skin disinfection methods. Transfusion 1997; 37:309–312 in Roback J ed. Technical Manual, 16th ed. Bethesda, MD. American Association of Blood Banks 2008:942–943

the donor. The phlebotomist must be aware of the needle placement to minimize the likelihood of puncturing a nerve or artery.

Blood collection

Usually the blood container is placed on a scale, which may have a device to cut off the flow when the container reaches a set weight indicating that the desired volume of blood has been collected. The blood must flow freely and be mixed with anticoagulant frequently as it fills the container to avoid the development of small clots. Some blood banks use mechanical devices that continuously mix the blood and anticoagulant during phlebotomy. No more than 15% of the donor's estimated blood volume should be collected and the limit of 10.5 mL/kg body weight [1] is intended to meet this limit. In addition, the volume of blood in the container should be between 405 and 550 mL (that is, 450 or 500 mL $\pm$ 10%). Thus, including specimens for testing, the amount of blood drawn could total 575 mL. Units containing 300–404 mL can be used for transfusion but must be labeled as low-volume units. The amount of blood withdrawn must be within prescribed limits to be in the proper ratio with the anticoagulant, otherwise the blood cells may be damaged or anticoagulation may not be satisfactory (see Chapter 5).

The actual time for phlebotomy and bleeding is usually about 7 minutes and almost always less than 10 minutes. If the blood flow is slow, clots may form in the tubing before the blood mixes with the anticoagulant in the container. Although there is no FDA-defined maximum allowable time for the collection of a unit of blood, most blood banks establish a maximum; usually no more than about 15 minutes. There is no difference in factor VIII or platelet recovery between units collected in less than 8 minutes versus those collected in 8–12 minutes [39]. Extremely rapid blood flow or the appearance of bright red blood may indicate an arterial puncture. This can be confirmed by feeling the pressure building in the blood container. An arterial puncture is nearly unmistakable because of the very rapid filling and pressure that develops in the blood container.

During blood donation, there is a slight decrease in systolic and a rise in diastolic blood pressure and peripheral resistance along with a slight fall in cardiac output but little change in heart rate [40]. During blood donation, the regional cerebral oxygen saturation decreases significantly but still remains within the range of individual physiologic variation while the cerebral tissue hemoglobin concentration increases significantly, probably due to an increase in cerebral blood volume which appears to be the major compensation mechanism during acute blood loss to maintain cerebral oxygenation [41].

At the conclusion of blood collection, the needle is removed and the donor is asked to apply pressure to the vein in the antecubital fossa for at least 1 or 2 minutes. Many blood centers have a policy of asking the donor to raise his or her arm to minimize the venous pressure while pressure is applied to the vein. When there is no bleeding, discoloration, or evidence of a hematoma at the venipuncture site, the donor should be evaluated for other symptoms of a reaction to donation. If none is present, the donor can move off the donor table to the refreshment area. The donor should be observed during this time, as the movement into an upright posture may bring on lightheadedness or even fainting.

4.3 Postdonation care and adverse reactions to blood donation

Postdonation care

Many donor reactions, especially lightheadedness or syncope, may occur when the donor is having refreshments. Donors are advised to drink extra fluids to replace lost blood volume. The nature of the fluid is not as important as consuming some fluid, except that alcoholic beverages are not recommended. The consumption of fluids helps to restore blood volume and minimize the postural hypotension that may occur for several hours after donation. Alcohol is a vasodilator and may cause a shift of blood flow to the periphery, resulting in reduced cerebral blood flow and hypotension or fainting. Even after the loss of a few hundred milliliters of blood, some donors are subject to lightheadedness or even fainting if they change position quickly. Therefore, donors are also advised not to return to work for the remainder of the day in an occupation where fainting would be hazardous to themselves or others. Donors are also advised to avoid strenuous exercise for the remainder of the day of donation [42]. This minimizes the chance of development of a hematoma at the venipuncture site and the chance of fainting due to diversion of blood from the central nervous system into peripheral vasculature.

Adverse reactions

A reaction occurs following approximately 4% of blood donations, but fortunately most reactions are not serious [43, 44]. Minimizing donor reactions begins with the selection of the site for blood collection, the staff training, the general treatment the donor receives from the staff, and the

Table 4.4 Adverse reactions to whole blood donation.

Hypovolemia	Syncope
	Lightheadedness
	Diaphoresis
	Nausea
	Vomiting
Vasovagal effects	Syncope
	Bradycardia
	Diaphoresis
	Pallor
Venipuncture	Hematoma
	Nerve injury
	Local infection
	Thrombophlebitis

ambience of the blood collection situation. These factors are important because reactions increase when the blood collection situation is crowded, noisy, or hot or when the donor endures a long wait. Donors who have reactions are more likely to be younger [45–47], to be unmarried, to have a higher predonation heart rate and lower diastolic blood pressure, and to be first-time donors or to have donated fewer times than donors who do not experience reactions [48].

Adverse reactions to blood donation can be categorized generally as those due to (a) hypovolemia, (b) vasovagal effects, and (c) complications of the venipuncture (Table 4.4). The most common symptoms of reaction to blood donation are weakness, cool skin, diaphoresis, and pallor. A more extensive but still moderate reaction may involve dizziness, pallor, hypertension, hypotension, and/or bradycardia. Bradycardia is usually taken as a sign of a vasovagal reaction rather than hypotensive or cardiovascular shock, in which tachycardia would be expected. In a more severe form, the reaction may progress to loss of consciousness, convulsions, and involuntary passage of urine or stool. The vasovagal syndrome can have important effects on blood donors and the blood supply [49]. The most common cause of these symptoms is probably due to the psychological stress of the situation or to neurologic factors rather than hypovolemia due to loss of blood volume. In the past, a common response to a donor reaction was to have the donor rebreathe into a paper bag. This is effective only if the lightheadedness is due to hyperventilation and reduced bicarbonate levels. Actually, most reactions do not have this basis and the paper bag may only add to the tension of the situation. This is not recommended as routine practice, but should be reserved for situations in which it seems clear that hyperventilation is a major part of the reaction. Other systemic reactions may include nausea, vomiting, and hyperventilation, sometimes leading to twitching or muscle spasms, convulsions, or serious cardiac difficulties. These kinds of serious reactions are very rare (see below). Several strategies that are being evaluated to

reduce donor reactions include predonation education, distraction, and additional water intake [50–52] which can reduce the rate of vasovagal reactions [52]. Minimizing donor reactions is very important because donors who experience adverse reactions are less likely to return [53].

No clinically significant effects have been reported for long-term multi-gallon donations of whole blood. However, some studies have reported immunologic abnormalities, including decreases in natural killer cells, total lymphocyte levels, and proliferative response to mitogens [54], although there were no differences between donors and nondonors in lymphocyte levels or function [54]. At present it is not considered that long-term blood donation adversely affects immunologic function.

Severe reactions to blood donation

Although most reactions are mild, severe reactions defined as those requiring hospitalization can occur. These include seizures, myocardial infarction, tetany, and death. Popovsky [44] reviewed 4,100,000 blood donations and found very severe reactions in 0.0005% or one per 198,110 allogeneic blood donations. The kinds of reactions included severe vasovagal reaction, angina, tetany, and problems related to the venipuncture site. Most reactions occurred during donation while the donor was at the donor site, although 6% occurred more than 3 days later. Reactions were more likely in first-time donors. If this incidence is generalized to the national blood collection activity of 12–14 million donations per year, approximately 60–70 such reactions may occur annually.

Seizures

Because seizures may occur following blood donation, a history of seizures has disqualified donors in the past. However, donors with a history of seizures well controlled at the time of donation have no greater likelihood of experiencing a reaction to donation than donors who had never had seizures [55]. Thus, blood banks may begin to modify their requirements regarding a history of seizures.

Nerve injuries

During venipuncture, the needle may accidentally strike a nerve. Injuries causing numbness or tingling, pain, and/or loss of arm or hand strength occur in approximately 1/21,000 to 26,700 donations [56, 57] and implies that approximately 500 to 600 such injuries may occur annually from the 12 to 14 million blood donations in the United States. Some of the donors developed a hematoma following donation, but it could not be determined whether the nerve damage was related to the hematoma or direct injury by the needle. One-third of the injuries resolved in less than 3 days, but 2% lasted longer than 6 months and 6% resulted in residual mild localized numbness [56].

In a detailed anatomic study of 11 patients with injury to upper extremity cutaneous nerves after routine venipuncture, Horowitz [58]

observed that nerve injury appeared secondary to direct trauma via "inappropriate" needle or bolused material near the nerves beneath the veins, or to nerves overlying the veins. However, in 3 of 13 additional patients, the venipunctures were properly performed and atraumatic. He explored the anatomic relationships of superficial veins and cutaneous nerves at three common venipuncture sites in the 14 upper extremities of seven randomly chosen cadavers. Major branches of cutaneous nerves were superficial to and overlay veins in 6 of the 14 extremities studied. In multiple instances, nerves and veins were intertwined. He concluded that anatomic relationships between upper extremity superficial veins and cutaneous nerves are so intimate that needle–nerve contact during venipuncture is common. Because venipuncture-induced nerve injuries are rare, factors other than direct nerve contact appear necessary for the chronic pain syndrome to occur.

Hematoma, arterial puncture, and thrombosis

A hematoma occurs commonly after blood donation even though the arm is inspected, and donors are advised to apply pressure to the area. Usually, these hematomas are not serious but they cause some local discoloration of the antecubital fossa. There may be some leakage of blood, which may soil the donor's clothes, resulting in the donor asking the blood center to pay for cleaning of the garment. A more serious but rare complication is the development of a large hematoma due to venous leakage from an arterial puncture. This can cause pressure on vessels or nerves in the antecubital fossa, and serious injury may result. Reports of symptoms suggesting this type of complication should be dealt with urgently by the blood center so that the donor can receive rapid attention and drainage of the fossa if necessary to prevent more serious injury. Although rare, deep vein thrombosis of the upper extremities has been reported as a complication of whole blood donation [59].

4.4 Therapeutic bleeding

Blood may be collected as part of the therapy of diseases such as polycythemia vera or hemochromatosis. Because the procedure is being performed as a therapy, these individuals are patients, not donors. Their medical assessment then is focused on determining that the phlebotomy is safe for the patient. The patients may meet all of the criteria for whole blood donation except for the presence of the disease for which they are undergoing phlebotomy. Often the patient or his or her physician asks that the blood be used for transfusion as a way of comforting the patient. However, usually blood collected as therapeutic bleeding is not used for transfusion, since the cause of the disease may not be known, and because of this the donors do not meet the FDA requirements. Possible blood donation by hemochromatosis patients was described previously in this chapter.

General assessment

The medical assessment and physical examination of potential cytapheresis donors is based on the effects of the procedure and potential complications. Donor selection and monitoring requirements for apheresis are designed to prevent the development of reactions or complications due to excess removal of blood cells or plasma.

The selection of donors for plateletpheresis, leukapheresis, and plasmapheresis uses the same general criteria used for whole blood donors [1]. Because of the unique nature of apheresis, there are some additional donor requirements that are based on the unique complications that may occur from apheresis, the nature of the procedures, and the fact that because few red cells are removed, donors can undergo cytapheresis more often than whole blood donation. The amount of blood components removed from apheresis donors must be monitored. To be consistent with whole blood donation, not more than 200 mL of red cells may be removed in 2 months [1]. If for some reason, such as instrument failure, it is not possible to return the red cells to the donor, then the donation is treated as if it were a whole blood donation, and the donor cannot donate again for 8 weeks. For consistency with plasma donation, not more than 1,000–1,200 mL of plasma per week may be retained [1]. When donors undergo apheresis more often than every 8 weeks, this is referred to as "serial" donation, and cumulative records must be maintained of the details of these donations and the records must be reviewed periodically by a physician. The laboratory testing of donors and apheresis components is the same as for whole blood donation. Thus, the likelihood of disease transmission from apheresis components is the same as for a component from whole blood.

Plateletpheresis donors

Plateletpheresis donors must meet the same medical requirements as whole blood donors. The platelet count decreases less than expected based on the number of platelets collected [60, 61] because platelets are mobilized during the apheresis procedure [62].

In one study [63] of 2069 plateletphereses in 352 donors, or an average of six procedures per donor, the following important observations were made that formed the basis of subsequent FDA regulations for the selection and monitoring of cytapheresis donors: (a) among women, platelet counts averaged 12% higher than those of males; (b) about 3% of all donors had platelet counts less than 150,000/mL before their first platelet donation; (c) the preapheresis platelet count was the best predictor of the postapheresis platelet count; (d) if donors with a preapheresis count of less than 150,000 were excluded, only 13% of donations resulted in a postapheresis count of less than 100,000; (e) the platelet count decreased about 30% immediately following apheresis; (f) the platelet count returned to normal about 4–6 days after apheresis; and (g) there was a slight

rebound in platelet count above the initial count about 8–11 days after apheresis. Although the decrease in platelet count varies somewhat with the procedure used, a decrease of 20–35% generally occurs and the platelet count returns to baseline levels about 4 days after donation [63].

Red cell loss
Collection of platelets, granulocytes, lymphocytes, or stem cells by cytapheresis results in very little red cell loss. Thus, red cell depletion is not considered a possible complication unless there is an instrument malfunction.

Blood volume shifts
Since no more than 15% of the donor's blood is extracorporeal at any time, there is no greater risk of blood volume shift than with whole blood donation. In addition, during apheresis, citrate and saline solutions are infused, replacing some of the lost blood volume. Thus, shifts in blood volume leading to hypotension are not a problem. Because of the administration of hydroxyethyl starch (HES) during leukapheresis, there was concern that a net increase in blood volume might occur because HES is used as a blood volume expander. This could lead to hypertension or acute heart failure. The volume of HES administered ranges from 200 to 400 mL and, combined with the removal of approximately 50–200 mL of granulocyte concentrate, does not result in complications due to excess blood volume.

Potential complications of serial donations
Because cytapheresis donors can donate more often than whole blood donors, there are some complications that could result from multiple frequent donations. These involve depletion of cells or plasma proteins.

Platelet depletion
Platelet depletion is a concern if donors undergo frequent plateletpheresis during a short period, although this was not observed in 352 donors who donated an average of six times [63].

A platelet count is not necessary before the initial donation because the decrease in platelet count following donation is not so extensive as to create a risk for the donor. At least 48 hours must elapse between platelet donations. If donors are to donate more frequently than every 4 weeks, a platelet count must be done to ensure that it is at least 150,000 per microliter before a subsequent donation [1]. The platelet count can be obtained before the donation, or a count obtained at the end of the previous donation can be used. Platelet donors should not have taken aspirin or drugs that interfere with platelet function within 3 days of donation.

Leukapheresis donors
Because the HES used in granulocyte collection is a blood volume expander, some blood banks use lower blood pressure levels than those

used for whole blood donors when selecting granulocyte donors. This is not a requirement, however. Granulocyte donors usually receive corticosteroids and many also receive granulocyte colony stimulating factor (G-CSF) to increase their granulocyte count and the granulocyte yield (see Chapter 7). Thus, donors should be questioned about conditions that might be exacerbated by corticosteroids. These include hypertension, peptic ulcers, and diabetes. Because corticosteroids are given to granulocyte donors, these donors usually do not donate frequently at short intervals. If this were to be done, it would need to be under the close supervision of a physician with written plans for monitoring the donor for side effects of accumulation of HES.

Plasmapheresis donors

If plasma is donated no more than every 8 weeks, the donor assessment procedures are the same as for whole blood. The FDA limitations for plasma removal are no more than 1,000–1,200 mL of plasma at one donation depending on the donor's weight [1]. These volumes may be slightly different when semiautomated instruments are used. Donors may give plasma more often than every 8 weeks, and this is called "serial" plasmapheresis. Donors may give again in 48 hours as for platelets but not more than twice within a 7-day period. For donors undergoing plasmapheresis more often than once every 4 weeks, the serum protein must be monitored and found to be within normal limits [1].

Donors for hematopoietic cell transplantation

Hematopoietic cell transplantation now uses a variety of donors such as unrelated marrow, peripheral blood stem cells (PBSC), or cord blood. Transfusion medicine physicians are involved in this donor selection process. The criteria for whole blood and apheresis donation serve as the basis for donor selection, but these criteria may be modified because the advantage of a particular donor may outweigh a very small or theoretical increased risk of the cellular product. Criteria intended to protect the donor are less likely to be modified but, as new donation situations such an unrelated marrow or cord blood have arisen, donor selection criteria for each situation were developed.

Physical examination of apheresis donors

The physical examination of cytapheresis donors is the same as for whole blood donation.

4.6 Adverse reactions in apheresis donors

General

Adverse reactions in apheresis donors (Table 4.5) are similar in character to those encountered in whole blood donation. Virtually all of the cytapheresis procedures carried out in normal donors are plateletpheresis. Normal donors undergoing plateletpheresis may report an adverse reaction

Table 4.5 Potential complications and adverse reactions to cytapheresis donation.

Reactions similar to whole blood donation
Citrate toxicity
Hematoma
Mechanical hemolysis
Air embolus
Platelet depletion
Lymphocyte depletion
Plasma protein depletion

following up to 50% of procedures when asked; however, such reactions cause the procedure to be discontinued in only 0.1–1.0% of the time [64]. These reactions are almost entirely due to citrate toxicity and can be alleviated by slowing the rate of blood return and thus the rate of citrate infusion.

Some potential complications of apheresis apply to all types of procedures because they have to do with the instrument or activities that are common to all types of procedures, while others are unique to certain apheresis procedures [65].

Vasovagal reactions

These reactions are similar to those associated with whole blood donation. The symptoms include weakness, pallor, diaphoresis, bradycardia, cold clammy skin, lightheadedness or fainting, and, if severe, convulsions. The treatment is as described for whole blood donors. Since the donors are usually experienced whole blood donors, they rarely experience vasovagal reactions.

Anticoagulation

The anticoagulant used for plateletpheresis is citrate. Cardiac toxicity due to calcium binding is a much more sensitive problem than in vivo anticoagulation (see below). Thus, bleeding due to citrate anticoagulation is not an issue.

Citrate toxicity

Elevations of blood citrate can cause paresthesias, muscle cramping, tetany, cardiac arrhythmia, and other symptoms. The plateletpheresis procedure involves the administration of citrate solutions to donors, almost as a form of massive autologous transfusion because 4–6 L of their blood is withdrawn, passed through the instrument, citrated, and returned to them during the procedure. During the development of apheresis techniques, there was a considerable interest in determining the particular citrate solution that would be optimal, the acceptable dose of citrate, and the nature and incidence of side effects. In a careful study relating the dose of citrate, symptoms, electrocardiographic changes, and ionized calcium, Olson et al. [66] showed that when citrate infusion rates were maintained

below 65 mg/kg/hr, donors did not experience symptoms nor demonstrate electrocardiographic abnormalities. This has been confirmed in later studies [67, 68]. Donors with similar levels of hypocalcemia may demonstrate wide variability in symptoms [69]. Using anticoagulant citrate dextrose (ACD) formula A at a ratio of 1 : 8 with whole blood, many abnormalities were observed including bradycardia, sometimes severe (less than 42); supraventricular and ventricular premature contractions; right bundle-branch block; ST segment elevation or depression; and tall, flattened, or inverted T waves. Some of the donors experienced nausea, vomiting, hypotension, fainting, or convulsions [70]. Even when less citrate is infused, the QT interval is almost always prolonged [71, 72]. Citrate reactions are managed by slowing the flow rate of the instrument and thus slowing the rate of citrate infusion. This is quite effective in eliminating these reactions, and most apheresis personnel are very aware of this process. Citrate toxicity can also occur if tubing is not properly placed in the pumps and the citrate solution is allowed to flow freely into the donor [73].

Circulatory effects

Because the extracorporeal volume of the cytapheresis instruments is small (usually less than 200 mL), hypovolemia is rare and these donors do not experience circulatory problems.

Air embolus

Because the blood is actively pumped into the donor's veins, there is the theoretical possibility that air could be pumped into the donor if air entered the system. Some of the early models of apheresis instruments contained bubble chambers connected to a device that stopped the pumps if the chamber became filled with air. Air emboli have occurred but no serious consequences have been reported. Contemporary instruments do not contain safety devices to prevent air embolus and so this complication remains a remote possibility. Staff members must be aware of this possibility and ensure that containers and tubing sets do not develop leaks that would allow air to enter the system.

Hematoma

Hematomas may develop after removal of the needles used for apheresis just as after whole blood donation. There is no reason that this should be a more or less frequent complication than following whole blood donation. However, because blood is returned to the donor by active pumping, if the needle becomes dislodged, the blood will continue to be injected into the antecubital fossa under pressure, and a substantial hematoma may develop quickly. The signs of this are pain, discoloration, or oozing at the venipuncture site. If this occurs, the blood flow is discontinued immediately, pressure is applied, and the hematoma is managed as described for whole blood donation.

Mechanical hemolysis

Because blood is pumped through tubing and centrifuges of various configurations, hemolysis is a theoretical complication due to constricted

tubing or the geometry of the flow pathways. Although these complications are rare, they have been reported to occur about 0.07% or once per about 1,500 procedures [74]. This means that a busy apheresis program would experience one or two incidents each year. This is a bit more frequently than these problems seem to occur in practice.

Platelet depletion or damage

Plateletpheresis does not damage the donor's remaining platelets, and the donor's platelet function is normal following donation [75]. Removal of platelets equivalent to several units of the donor's blood does not result in thrombocytopenia. In a rather dramatic example, a female donor underwent 101 donations during a 33-month period, with donation frequencies ranging from once to three times weekly [76]. Her platelet count remained in the range of 135,000–430,000 during this time. In donors who undergo repeated plateletpheresis, the platelet count decreases somewhat more but then stabilizes [63,77]. Thus, it appears that platelets can be donated safely approximately every 2–4 days.

Lymphocyte depletion

Because a relatively large number of lymphocytes are removed during plateletpheresis using early models of apheresis instruments, concern developed about the possibility of lymphocyte depletion and altered immunologic status in normal donors undergoing frequent plateletpheresis. Senhauser et al. [78] found a 23% decrease in total lymphocyte count, a 25% decrease in T cells, and a 47% decrease in B cells in donors who underwent 9 plateletphereses in 1 year compared with those who gave 1–4 units of whole blood in the same time. Koepke et al. [79] also found a 20% decrease in total lymphocyte count and a substantial decrease in B cells in frequent cytapheresis donors. In another study of 25 volunteers who underwent an average of 72 platelet donations during about 8 years, there was a decrease in total lymphocytes, T4 cells, T4/T8 ratio, and response to mitogen stimulation [80]. Wright et al. [81] studied patients (not normal donors) who underwent a 4-hour lymphapheresis two to three times per week for 5–7 weeks (for a total of 13 to 18 procedures). Each procedure involved removal of approximately 3.5×10^9 lymphocytes, or up to 2.5×10^{10} lymphocytes per week. A fall in lymphocyte count of about 25% occurred after the first three procedures, and the count remained stable after the second week. The study established that at least 10^9 lymphocytes must be removed daily for several days for a measurable decline in lymphocyte count to occur. At least 10^{11} lymphocytes must be removed over a short time and/or the individual's lymphocyte count must be less than 500 per microliter for clinical immunosuppression to occur [82]. Although lymphocyte depletion can occur with repeated lymphapheresis, the number of lymphocytes removed during ordinary plateletpheresis, even on multiple occasions during a relatively short period of time, is not a clinical risk for normal donors.

During the past few years, instruments and procedures have been adjusted to minimize the leukocyte content. As a result, most plateletpheresis procedures today remove about 1×10^6 to 5×10^7

leukocytes. Loss of this number of leukocytes is very unlikely to lead to leukocyte depletion or any clinical effects on the donor's immune function.

Complications unique to leukapheresis

The complications related to leukapheresis are usually not different from those in plateletpheresis except that donors receive HES, corticosteroids, and possibly G-CSF. There are only a few studies of frequent granulocyte donors. Strauss et al. [83] reported 13 donors who gave between 12 and 29 times on consecutive days. Platelet and hemoglobin levels remained unchanged, but leukocyte levels decreased. Some donors had skin rashes, probably due to the HES. Hypertension and peripheral edema are potential complications from fluid accumulation caused by the HES. Side effects of corticosteroids are well known and those related to G-CSF are discussed in Chapter 17. It has been suggested that frequent administration of corticosteroids to granulocyte donors may lead to cataract formation [84], but this was not confirmed in a subsequent study [85].

Complications unique to plasmapheresis

When plasmapheresis was done with plastic bags, there was a risk of returning the red cells to the wrong donor. The use of semiautomated instruments has eliminated this risk. Depletion of plasma proteins is avoided by monitoring these levels in the donor. One unexpected risk is the development of anemia, probably due to the blood samples used for laboratory testing [86].

Complications unique to mononuclear cell apheresis for collection of peripheral blood stem cells

Because of the low level of circulating PBSCs in normal donors, donors receive the hematopoietic growth factor G-CSF to mobilize PBSCs and increase the yield (see Chapter 17). G-CSF is associated with a rather high frequency of side effects. Thus, virtually all of the complications and side effects of the donation of mononuclear cells as a source of PBSCs are related to the administration of the G-CSF [87].

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5 Preparation, Storage, and Characteristics of Blood Components and Plasma Derivatives

In the United States and developed countries, whole blood is rarely used. Within a few hours or days, some coagulation factors, especially V and VIII, and platelets decrease in quantity or lose viability in stored whole blood. Therefore, virtually all whole blood collected is separated into its components, and each component is stored under conditions optimal for that component. This makes it possible to retain all of the activities of the original unit of whole blood and results in a large number of different components being available for transfusion therapy (Tables 5.1 and 5.2). A few physicians continue to use whole blood for selected indications such as cardiovascular surgery and exchange transfusion of the neonate; however, it is usually not possible to provide whole blood of less than 48-hour storage due to the time necessary to complete testing. In contrast, it is possible to provide, through a combination of blood components, everything that can be provided in fresh whole blood. It is difficult to resolve the value of fresh whole blood by clinical trials because the blood banking system in the United States is so structured to produce components. In less developed parts of the world, whole blood is usually used because equipment to produce components may not be available. However, in these situations, given the kinds of patients being transfused, whole blood may be the best use of limited blood transfusion resources. In addition, the use of rapid tests for HIV, HCV, HBV, and syphilis make fresh whole blood available.

This chapter describes the characteristics of blood products, their preparation, and storage. Clinical uses are described in Chapters 11 and 12.

5.1 Preparation of blood components from whole blood

Anticoagulant–preservative solutions

The beginning of red cell preservation can be traced to Peyton Rous, who later was awarded the Nobel prize for his work with viruses. Rous and

Table 5.1 Components produced by blood banks and the medical use of these components.

Component	Medical use
Red blood cells	Oxygenation of tissues
Platelets	Prevention or cessation of bleeding
Fresh frozen plasma	Cessation of bleeding
Cryoprecipitate	Cessation of bleeding
Cryoprecipitate-poor plasma	Plasma exchange
Granulocytes	Treatment of infection
Frozen red blood cells	Storage of rare blood
Leukocyte-depleted red cells	Prevention of reactions and certain diseases

Table 5.2 Products produced from whole blood subject to licensure by the FDA.

Red cell components	Red blood cells
	Red blood cells deglycerolized
	Red blood cells deglycerolized irradiated
	Red blood cells frozen
	Red blood cells frozen irradiated
	Red blood cells frozen rejuvenated
	Red blood cells frozen rejuvenated irradiated
	Red blood cells irradiated
	Red blood cells leukocytes removed
	Red blood cells leukocytes removed irradiated
	Red blood cells rejuvenated
	Red blood cells rejuvenated deglycerolized
	Red blood cells rejuvenated deglycerolized irradiated
	Red blood cells rejuvenated irradiated
	Red blood cells washed
	Whole blood CPD irradiated
	Whole blood cryoprecipitate removed
	Whole blood leukocytes removed
	Whole blood modified—platelets removed
	Whole blood platelets removed irradiated
Plasma components	Cryoprecipitate AHF
	Cryoprecipitate AHF irradiated
	Cryoprecipitate AHF pooled
	Fresh frozen plasma
	Fresh frozen plasma irradiated
	Liquid plasma
	Plasma
	Plasma irradiated
Platelets	Platelet-rich plasma
	Platelets
	Platelets irradiated
	Platelets pheresis
	Platelets pheresis irradiated

AHF, antihemophilic factor;
CPD, citrate–phosphate–dextrose; FDA, Food and Drug Administration.

Table 5.3 Content of anticoagulant–preservative solutions (mg in 63 mL).

	CPD	CP2D	CPDA-1	ACD-A	ACD-B
Sodium citrate	1660	1660	1660	1386	832
Citric acid	206	206	206	504	504
Dextrose	1610	3220	2010	1599	956
Monobasic sodium phosphate	140	140	140	–	–
Adenine	0	0	17.3	–	–

Source: Roback J ed. Technical Manual, 16th ed. Bethesda, MD. American Association of Blood Banks, 2008, p. 190.
CACD, acid–citrate–dextrose; PD, citrate–phosphate–dextrose.

Turner [1] showed that glucose delayed in vitro hemolysis. During the period between World Wars I and II, Mollison [2] in England developed an acidified citrate and glucose solution for red cell preservation, variants of which are the mainstay of present-day preservatives. These solutions are composed of citrate for anticoagulation, dextrose for cell maintenance, and phosphate buffers (Table 5.3). Whole blood or red blood cells can be stored in these solutions for periods ranging from 21 to 35 days.

During red cell preservation, adenosine triphosphate (ATP) loss correlates with poor red cell viability, and addition of adenine at the beginning of preservation increases ATP and improves red cell viability [3]. 2,3-Diphosphoglycerate (2,3-DPG) also declines in stored red cells and this is associated with increased affinity of hemoglobin for oxygen [4–6]. Thus, there was considerable interest in developing solutions that would maintain both ATP and 2,3-DPG while allowing removal of the maximum volume of plasma for production of derivatives (Table 5.4). During the past few years, it has been possible to extend the duration of red cell storage by placing the red cells in special "additive" solutions containing various combinations of saline, adenine, phosphate, bicarbonate, glucose, and mannitol [7–10]. These solutions provide better nutrients that maintain red cell viability, red cell enzymes, and red cell function, allowing red cell preservation for 42 days.

Blood processing for the preparation of components

Because most blood is separated into its components, the whole blood is collected into sets involving multiple connected bags. The blood first enters the primary bag, where it is mixed with anticoagulant–preservative solution. After collection, the whole blood is kept at a temperature either between 1°C and 6°C or between 20°C and 24°C depending on the intended use of the unit (Figure 5.1). If red blood cells and plasma are to be produced, the blood is kept between 1°C and 6°C. When the blood is collected in a fixed site, the blood can be placed in a regular blood storage refrigerator. If the collection is at a mobile site, special insulated containers are used that contain ice and maintain that temperature. If platelets are to be prepared from the whole blood, the blood must be maintained at room temperature (20–24°C) because exposure to cold damages the platelets.

Table 5.4 Plasma-derivative products.

Albumin	Restoration of plasma volume subsequent to shock, trauma, surgery, and burns
Alpha1 proteinase inhibitor	Used in the treatment of emphysema caused by a genetic deficiency
Antihemophilic factor	Treatment or prevention of bleeding in patients with hemophilia A
Anti-inhibitor coagulant	Treatment of bleeding episodes in the presence of complex factor VIII inhibitor
Antithrombin III	Treatment of bleeding episodes associated with liver disease, Antithrombin III deficiency, and thromboembolism
Cytomegalovirus immune	Passive immunization subsequent to exposure to globulin cytomegalovirus
Factor IX complex	Prophylaxis and treatment of hemophilia B bleeding episodes and other bleeding disorders
Factor XIII	Treatment of bleeding and disorders of wound healing due to factor XIII deficiency
Fibrinogen	Treatment of hemorrhagic diathesis in hypofibrinogenemia, dysfibrinogenemia, and afibrinogenemia
Fibrinolysin	Dissolution of intravascular clots
Haptoglobin	Supportive therapy in viral hepatitis and pernicious anemia
Hepatitis B immune globulin	Passive immunization subsequent to exposure to hepatitis B
IgM-enriched immune globulin	Treatment and prevention of septicemia and septic shock due to toxin liberation in the course of antibiotic treatment
Immune globulin (intravenous and intramuscular)	Treatment of agammaglobulinemia and hypogammaglobulinemia; passive immunization for hepatitis A and measles
Plasma proteinfraction	Restoration of plasma volume subsequent to shock, trauma, surgery, and burns
Rabies immune globulin	Passive immunization subsequent to exposure to rabies
RhO(D) immune globulin	Treatment and prevention of hemolytic disease of fetus and newborn resulting from Rh incompatibility and incompatible blood transfusions
Rubella immune globulin	Passive immunization subsequent to exposure to German measles
Serum cholinesterase	Treatment of prolonged apnea after administration of succinyl choline chloride
Tetanus immune globulin	Passive immunization subsequent to exposure to tetanus
Vaccinia immune globulin	Passive immunization subsequent to exposure to smallpox
Varicella-zoster immune	Passive immunization subsequent to exposure to globulin chicken pox
von Willebrand factor/factor VIII concentrate	Treatment or prevention of bleeding in von Willebrand's patients

Source: From information provided by the American Blood Resources Association.

Table 5.5 Content of additive solutions.

	AS-1 (Adsol)	AS-3 (Nutricel)
Dextrose	2200	1100
Adenine	27	30
Monobasic sodium phosphate	0	276
Mannitol	750	0
Sodium chloride	900	410
Sodium citrate	0	588
Citric acid	0	42

Source: Roback J, ed. Technical Manual, 16th ed. Bethesda, MD: American Association of Blood Banks, 2008, p. 191.

Maintenance of the blood at room temperature is done by placing the units in containers specially designed to maintain that temperature. It is recognized that the blood will not attain the temperatures of the storage containers (1–6°C or 20–24°C) for several hours, but the blood must be placed in the environment that will begin to bring the temperature of the blood to the desired storage temperature.

To prepare the components, large high-speed centrifuges that accommodate four or six units of whole blood are used. Important factors in the centrifuge techniques include the rotor size, centrifuge speed, time at maximum speed, and braking mechanism or deceleration phase. The whole blood is manipulated differently depending upon the components desired (see below). After removal of the platelet-rich plasma, the additive solution is added to the concentrated red cells for optimum red cell preservation (Figure 5.2).

	Primary container	Container with additive solution	Transfer pack
Original contents:	Anticoagulant solution	Additive solution	Empty
Final contents:	Red blood cells	Plasma	Platelet concentrate

To donor phlebotomy site
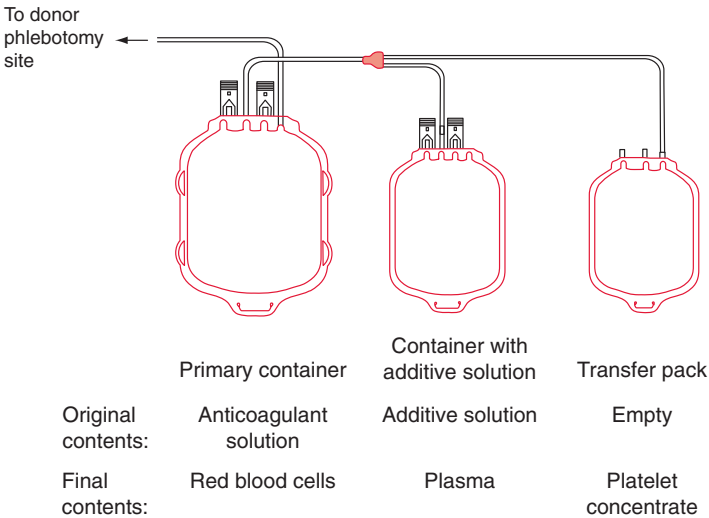
Figure 5.1 Diagram showing unit of whole blood and integral plastic bag system used for preparing blood components.
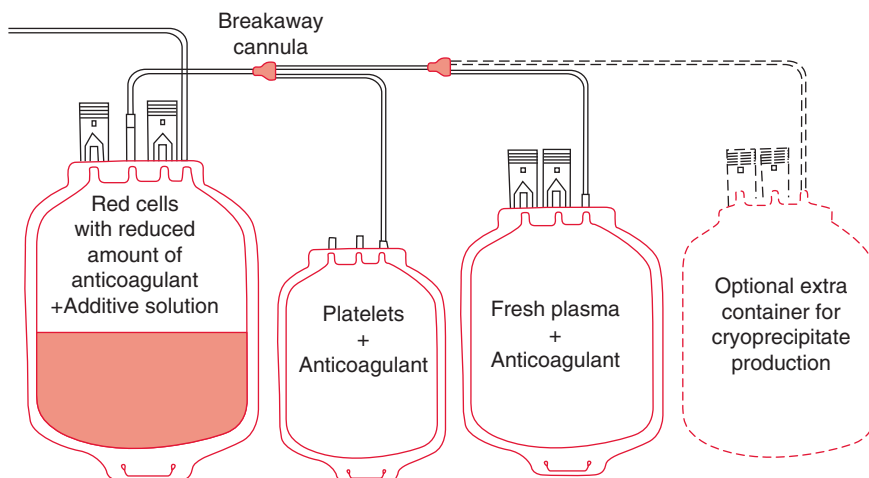
Figure 5.2 Diagrammatic illustration of the separation of whole blood into red cells, plasma, and platelet concentrate.

Red blood cells
Description of component
Red blood cells are the cells remaining after most of the plasma has been removed from whole blood. They must have a final hematocrit less than 80% [11]. This blood component is often called "packed red cells" or "packed cells." Usually the red cells and plasma are separated within 8 hours of collection because for the plasma to be used as a source of factor VIII it must be placed in the freezer before 8 hours after collection. Centrifugation is used when the red cells are being prepared within a few hours after collection, because usually this is done as part of a large-scale operation, and speed is important. Usually the unit of whole blood is centrifuged to produce a platelet concentrate and/or to recover plasma. Therefore, the centrifugation conditions (time and speed) are determined by the method being used to prepare the platelets or plasma. If platelets or fresh frozen plasma (FFP) are not being produced from the original unit of whole blood, the red cells can be separated from the plasma at any time during the storage period of the blood. If the unit of whole blood is allowed to remain undisturbed for several hours, the red cells sediment and the plasma can be removed. When sedimentation is used, the red cells are not as concentrated; as a result, the red cell unit has a lower hematocrit and less plasma is recovered. Because the plasma is valuable as a source for production of plasma derivatives, it is desirable to recover the maximum amount of plasma, and therefore, sedimentation currently is not usually used to separate whole blood into its components. However, sedimentation can be used quite effectively when equipment for centrifugation is not available such as in developing countries.

The unit of red cells has a volume of about 300 mL and will contain a minimum of 154 mL of red cells (405 mL × 38% hematocrit). Usually the

Table 5.6 Characteristics of red cells in AS-1 (Adsol) for 42 days of storage[a].

Indices	Days of storage						
	0	7	14	21	28	35	42
pH (at 37°C)	7.00	6.86	6.69	6.55	–	6.43	3.34
RBC ATP (mmol/g Hgb)	4.69	4.97	4.83	4.50	3.75	3.47	3.24
RBC DPG (mmol/g Hgb)	10.88	8.16	1.96	0.87	0.65	0.54	0.65
Supernatant sodium (mEq/L)	152	135	131	124	–	126	123
Supernatant potassium (mEq/L)	1.6	17	27	34	–	44	46
Supernatant glucose (mg/dL)	909	780	724	697	660	617	604
Hemolysis (%)	0.02	0.06	0.11	0.14	0.20	0.16	0.24

Source: Reproduced with permission from Fenwal with permission McCullough J. Transfusion medicine. In: Handin RI, Lux SE, Stossel TP, eds. Blood: Principles and Practice of Hematology. Philadelphia: Lippincott, Williams and Wilkins, 2003, p. 2018.
[a]AS-1 cells: N = 13; volume = 325 + 29 mL; hematocrit = 58% + 4%; mean red cell mass = 188 mL; mean supernatant volume = 136 mL; mean total hemoglobin = 19.3 g%.

red cell unit contains about 190 mL of red cells (450 mL × 42% hematocrit) and has a hematocrit of about 60%. The fluid portion of the unit (approximately 130 mL) is primarily the additive preservative solution, although about 20 mL of plasma remains from the original unit of whole blood. The characteristics of a unit of red cells stored in an additive are illustrated in Table 5.6 and the general changes in Table 5.7. One unit of red cells will increase the hemoglobin concentration and hematocrit in an average-sized adult (70 kg) by about 1 g/dL and 3%, respectively.

Storage conditions and duration
Red cells are stored at 1–6°C for 21–42 days depending on the anticoagulant–preservative solution used. For optimum inventory management, most blood centers use preservatives that allow 42-day storage. The end of the storage period is referred to as the expiration date or the "outdate." The cells must be stored in refrigerators with good air circulation and that are designed for blood storage. Household

Table 5.7 Changes occurring during red cell storage: the storage lesion.

Increase	Decrease
Lactate	ATP
Pyruvate	2,3-DPG
Ammonia	Intracellular potassium
Intracellular sodium	PH
Membrane vesicles	
Plasma hemoglobin	

refrigerators are not suitable. The temperature in the refrigerator must be monitored and should be recorded periodically (continuously, if possible), at least every 4 hours. There should be an alarm system to warn staff if the temperature moves outside the acceptable limits. When blood is transported to the patient care area for transfusion, it may be allowed to warm to 10°C and still be suitable for return to the blood bank and reissue to other patients [11].

Blood components must be maintained under proper storage conditions during transportation from the blood center to the hospital transfusion service. Various containers are available for this purpose, and these processes are standard and work well in developed countries. However, in developing or undeveloped parts of the world usually these kinds of containers are not available and red cells may not be refrigerated or stored properly during this transportation. This is also an issue in military settings where it also important that these containers be lightweight. At least one container has been reported that will maintain red cells at 1–10°C for up to 78 hours [12].

Frozen or deglycerolized red blood cells
Description of component

Red blood cells, frozen or deglycerolized, are the cells that have been stored in the frozen state at optimal temperatures in the presence of a cryoprotective agent, which is removed by washing before transfusion [11, 13]. The red cells must be frozen within 6 days after collection, and they can be stored for up to 10 years, although the AABB Standards [11] do not include a standard for storage duration and acceptable post-thaw results have been found after storage at −80°C for 37 years [14–17]. The cryoprotectant commonly used is glycerol, which must be removed before transfusion in order to avoid osmotic hemolysis when the cells are transfused. The method of freezing and storage must preserve at least 80% of the original red cells, and at least 70% of those cells must survive 24 hours after transfusion.

Freezing of red cells is based on work from almost 50 years ago showing that glycerol protected human red cells from freezing injury [18] and that red cells preserved with glycerol were clinically effective [19–21]. From this work, "high-" and "low-" concentration glycerol methods were developed [22, 23]. These methods actually relate to the rate of freezing, which determines the nature of the freezing injury to the cells. When freezing is slow, extracellular ice forms, which increases the extracellular osmolarity, causing intracellular water to diffuse out of the cell and resulting in intracellular dehydration and damage [22]. This type of injury is prevented by solutes such as glycerol that penetrate the cell and minimize the dehydration [22]. Because the freezing process is slow, high concentrations of cryoprotectant, usually 40% glycerol, are required. Red cells preserved with this high concentration of glycerol can be stored at about −85°C, a temperature that is achievable by mechanical freezers.

Rapid freezing causes intracellular ice crystals and resulting cell damage [22]. However, because the freezing is faster, lower concentrations of

cryoprotectant, usually about 20% glycerol, are effective [24]. This lower concentration of glycerol necessitates storage of red cells at a temperature of about −196°C, achievable only by using liquid nitrogen.

These two methods—with different freezing rates, concentrations of glycerol, storage conditions, and processes for removing the glycerol—involve different technologies [22, 24]. Technology development played a major role in making red cell freezing clinically available. During the 1970s, a disposable plastic bowl and semiautomated washing system were developed that greatly facilitated glycerol removal from high glycerol concentration red cells [25–27]. A separate system using sugars to agglomerate red cells and allow washing to remove glycerol was used for a time [28], but it was replaced by more simple semiautomated techniques. In the rapid-freeze method, the concentration of glycerol is low enough that glycerol removal can be done by washing in ordinary blood bags; complex instruments are not required. To summarize, the high-concentration glycerol method involves more simple freezing and storage but complex deglycerolizing procedures. The low-concentration glycerol method involves complex freezing and storage but simple deglycerolizing procedures. Frozen deglycerolized red cells are composed of essentially pure red cells suspended in an electrolyte solution (Table 5.8). Most of the plasma, platelets, and leukocytes have been removed either by the freezing, thawing, or washing step necessary to remove the glycerol cryoprotectant. Thus, the deglycerolized red cells have a 24-hour storage period, which is a major factor in the logistics of their use.

Red cells must be frozen within 6 days of collection to provide acceptable post-transfusion survival. Red cells that have been stored longer than 6 days can be frozen if they are "rejuvenated" [29]. Rejuvenation

Table 5.8 General comparison of methods of preparing leukocyte-depleted red cells for transfusion.

Method	White cells removed (%)	Approximate number of WBCs remaining	Original red cells remaining (%)	Comment
Centrifugation	85	1×10^9	80	Product may contain dextran or hydroxyethyl starch; 24-h storage
Sedimentation (dextran)	95	0.9×10^9	90	24-h storage; product may contain dextran or hydroxyethyl starch
Freezing deglycerolizing	98	0.1×10^9	90	24-h storage
Washing	85	0.1×10^9	85	24-h storage
Spin cool filter	90	0.3×10^9	90	24-h storage
Nylon filter	65	1.5×10^9	88	Heparin required; 24-h storage
New-generation filters[a]	99	5×10^6	95	Bedside use

Source: Reproduced with permission from McCullough J. Transfusion medicine. In: Handin RI, Lux SE, Stossel TP, eds. Blood: Principles and Practice of Hematology. Philadelphia: J.B. Lippincott, 2003, pp. 2011–2068.
[a]Refers to general results from several different manufacturers' filters that have become available during the late 1980s.

restores metabolic functions after the red cells are incubated with solutions containing pyruvate, inosine, glucose, phosphate, and adenine followed by freezing [29]. This is a helpful strategy to freeze red cells in situations such as (a) red cells found after 6 days of storage to have a very rare phenotype, (b) red cells donated for autologous transfusion but the surgery is postponed, and (c) rare phenotype red cells thawed but not used. The rejuvenation and subsequent freezing process is complex and expensive and is not widely used.

Washed red cells

The definition of washed red cells is rather vague. These are the red cells remaining after washing with a solution that will remove almost all of the plasma [11]. Thus, the requirements for this component do not specify the nature of the washing solution or the exact composition of the final component. Red cells can be washed by adding saline to the red cells in an ordinary bag, centrifuging them and removing the supernatant, or by using semiautomated washing devices such as those used for deglycerolization [30–32]. Depending on the solution and technique used, the washed red cells may have a variable content of leukocytes and platelets. There is usually some red cell loss during the washing step, and the resulting red cell unit may contain a smaller dose of red cells than a standard unit. In general, the characteristics of washed red cells are the removal of approximately 85% of the leukocytes, loss of about 15% of the red cells, and loss of more than 99% of the original plasma [30–32]. Because the washing usually involves entering the storage container, the washed red cells have a storage period of 24 hours.

Leukocyte-reduced red blood cells

Definition of component

Leukocyte-reduced red cells are cells prepared by a method known to retain at least 80% of the original red cells and reduce the leukocyte content to less than 5×10^6 [11].

History of leukodepletion

The blood filters routinely used for routine transfusions have a pore size of 170–260 mm. They filter out clots and fibrin strands but do not effectively remove leukocytes. During the 1960s and 1970s, it was recognized that leukocytes were important in the pathogenesis of febrile nonhemolytic transfusion reactions and could cause alloimmunization, which would later interfere with organ transplantation or platelet transfusion (see Chapter 14). Thus, considerable interest developed in removing leukocytes before transfusion. Early methods involved centrifuging the red cells (either upright or inverted); sedimenting red cells with dextran or hydroxyethyl starch; filtration with nylon or cotton wool, which removed only granulocytes [33]; or washing, freezing, and deglycerolizing [32]. These methods removed from 65% to 99% of the original leukocytes and from 5% to 20% of the original red cells. A huge body of literature developed describing the advantages and disadvantages of the different methods and some of the clinical effects of their use. Although they are of

Table 5.9 Established or potential adverse effects of leukocytes in blood components.

Immunologic effects	Alloimmunization
	Febrile nonhemolytic transfusion reactions
	Refractoriness to platelet transfusion
	Rejection of transplanted organs
	Graft-versus-host disease
	Transfusion-related acute lung injury
Immunomodulation	Increased bacterial infections
	Increased recurrence of malignancy
Infectious disease transmission	Cytomegalovirus infection
	HTLV-I infection
	Epstein–Barr virus infection

historical interest, these studies are not described extensively here because the methods are not used today. Leukodepletion continues to be a very major issue in transfusion medicine because even more adverse effects of leukocytes contained in blood components have been identified (Table 5.9). The consequences of these effects are described in more detail in Chapter 14. As these adverse effects of leukocytes have been more extensively described, the technology for producing leukodepleted red cells has evolved to provide more extensive leukocyte depletion than was possible using earlier methods [34].

Clinical and animal studies suggested that red cells intended to prevent febrile nonhemolytic transfusion reactions must contain fewer than 5×10^8 leukocytes, and those intended to prevent alloimmunization contain fewer than 5×10^6 leukocytes [11]. The latter requires removal of about 99.9% of the leukocytes. Sophisticated filters have been developed to accomplish this.

Leukocyte depletion filters

The filter material may be modified to alter the surface charge and improve the effectiveness. The mechanism of leukocyte removal by the filters currently in use is probably a combination of physical or barrier retention and also biological processes involving cell adhesion to the filter material. These are sometimes referred to as 3 log 10, 3 log, or third-generation filters because they remove more than 99.9% of the leukocytes. With the development of these new, very effective filters, several procedural issues have surfaced.

Because leukocytes are contained in red cell and platelet components, filters have been developed for both of these components. Filters are available as part of multiple-bag systems including additive solutions so that leukocytes can be removed soon after collection and the unit of whole blood converted into the usual components. Filtration removes 99.9% of the leukocytes along with a loss of 15–23% of the red cells [35]. Filters fail to achieve the desired leukodepletion from 0.3% to 2.7% of units. Red cell components from donors with sickle cell trait often occlude white cell reduction filters.

It appears that febrile nonhemolytic transfusion reactions are caused not only by leukocyte antigen–antibody reactions but also by the cytokines produced by leukocytes in the transfused blood component (see Chapter 14). This would be more effectively prevented if the leukocytes were removed immediately after the blood is collected, avoiding the formation of cytokines. This is referred to as "prestorage" leukodepletion. Filtering the red cells at the bedside at the time of transfusion has the advantage of not requiring a separate blood bank inventory, but the disadvantage of allowing cytokines to accumulate during blood storage, thus being less effective in preventing febrile transfusion reactions (see Chapter 14). Also, bedside filtration is not as effective in removing leukocytes as filtration in the laboratory under standardized conditions and with a good quality control program [35]. Because the leukocyte content of the depleted units is very low, the usual methods for leukocyte counting are not accurate [35–37]. The Nageotte chamber and flow cytometry are used for this quality control testing [35, 37].

Fresh frozen plasma
Description of component
FFP is plasma separated from whole blood and placed at −18°C or lower within 8 hours of collection [11]. Fresh plasma may be frozen by placing it in a liquid freezing bath composed of ethanol and dry ice, or between blocks of dry ice, or in a mechanical or a blast freezer. The unit of FFP has a volume of about 200–250 mL and contains all of the coagulation factors present in fresh blood. FFP can also be produced as a byproduct of plateletpheresis, and this may result in a unit of FFP with a volume of about 500 mL, often called "jumbo" units of FFP. These can be stored or separated into two separate products. The electrolyte composition of FFP is that of freshly collected blood and the anticoagulant solution. FFP is not considered to contain red cells, and so is usually administered without regard to Rh type. However, there have been occasional rare reports suggesting that units of FFP contain a small amount of red cell stroma that can cause immunization to red cells [38]. Because it contains ABO antibodies, the plasma must be compatible with the recipient's red cells. The number of leukocytes in FFP depends on the centrifugation procedures used for preparation.

Storage conditions and duration
FFP is stored at −18°C or below and can be stored for up to 1 year after the unit of blood was collected. Although there is no defined lower temperature for FFP storage, freezers capable of maintaining very low temperatures such as −65°C are not usually used for storage of FFP because these freezers are expensive to operate and there is no reason to keep the FFP that cold.

24-Hour frozen plasma (FP24)
This is plasma frozen more than 8 and less than 24 hours after collection. It contains normal amounts of factor V but only an average amount of about

55–75% of factor VIII [13]. FP24 is often used interchangeably with FFP (see Chapter 11).

Thawing

FFP or FP24 are usually thawed in a 37°C water bath in an overwrap. However, it takes about 15–20 minutes to thaw one or two units, and someone must occasionally manipulate the unit to speed thawing by breaking up the pieces of ice. This time requirement is a substantial difficulty when FFP is needed for actively bleeding patients. Several approaches have been used, including larger water baths and water baths with agitating trays so that staff members do not need to manipulate the units. Another more promising approach is the use of microwave ovens for thawing FFP [39, 40]. The concern with microwave thawing has been the uneven energy distribution within the unit of FFP and resulting "hot spots" and damage to proteins. As microwave devices have improved, it appears that these problems have been overcome. After thawing, FFP or FP24 can be stored for 4 days at 1–6°C. Upon thawing, factor VIII and protein C levels are lower in FP24 than FFP, but there are adequate coagulation factor activities to maintain hemostatic effectiveness [41]. By day 5, factor V levels are about 60% and factor VIII levels 40–70%. Thus, as a source of coagulation factor replacement, it is best to use the thawed plasma within 24–48 hours. This does, however, make it possible to maintain a stock supply of thawed plasma for emergency use in massive transfusion or urgent correction of warfarin therapy.

Plasma
Description of component

Plasma from a unit of whole blood can be removed at any time during the storage period of the whole blood unit or up to 5 days after the unit outdates. This plasma can be stored for up to 5 years at −18°C or lower. Because it was not frozen within 8 hours after the whole blood was collected, plasma is not a satisfactory source of coagulation factors V and VIII. Although other coagulation factors are present because the plasma is removed from the whole blood after several days of storage, the electrolyte concentrations of the plasma will reflect those of stored whole blood. Thus, plasma is not usually used for transfusion.

Cryoprecipitate
Description of component

Coagulation factor VIII is a cold insoluble protein [42]. Pool and Shannon [43] took advantage of this to develop a method to recover most of the factor VIII from a unit of whole blood in a concentrated form. Cryoprecipitate is the cold insoluble portion of FFP that has been thawed between 1°C and 6°C. The cold insoluble material is separated from the thawed plasma immediately and refrozen within 1 hour. Although there are no specific requirements for the volume of a unit of cryoprecipitate, it is usually 5 mL or more, but less than 10 mL. The cryoprecipitate units must contain at least 80 units of factor VIII and 150 mg of fibrinogen [11].

Cryoprecipitate is not a suitable source of coagulation factors II, V, VII, IX, X, XI, and XII [44]. Several factors influence the content of factor VIII in cryoprecipitate, including the blood group of the donor (group A higher than group O), the anticoagulant (citrate–phosphate–dextrose (CPD) higher than acid–citrate–dextrose (ACD)), the age of the plasma when frozen, and the speed of thawing the FFP [45]. Cryoprecipitate also contains fibrinogen and von Willebrand factor. Each bag of cryoprecipitate contains about 250 mg of fibrinogen [44].

Cryoprecipitate is stored at $-18°C$ or below and can be kept for up to 1 year.

Thawing
Cryoprecipitate is thawed at $37°C$, usually in a water bath. Care must be taken to ensure that the water bath is not contaminated and that the bags of cryoprecipitate are placed inside another bag (overwrap) to minimize the chance of contamination. When using cryoprecipitate, it is customary to pool several bags so that only one container is sent to the patient care area for transfusion. Some blood centers pool several bags of cryoprecipitate before freezing them; this is more convenient for the transfusion service because it eliminates the need to pool individual bags of cryoprecipitate after they are thawed. After thawing, the cryoprecipitate must be maintained at room temperature to avoid reprecipitation of the factor VIII.

Platelet concentrates—whole blood
Description of component
The official term for this component is platelets. These are platelets suspended in plasma or platelet additive solution (Table 5.10) prepared by centrifugation of whole blood. They are also often referred to as "random-donor" platelet concentrates. Platelets may also be produced by cytapheresis (see Chapter 7). A unit of whole-blood-derived platelets must contain at least 5.5×10^{10} platelets [11]. Although there is no required

Table 5.10 Content of platelet additive solutions.

	InterSol[a] (Fenwal)	Composol (Fresenius)	Plasma Lyte A (Baxter)	SSP+ (MacoPharma)
Sodium chloride	77	90	90	69
Potassium chloride	0	5	5	5
Magnesium chloride	0	1.5	3	1.5
Sodium citrate	10	11	0	10
Sodium phosphate buffer	26	0	0	26
Sodium acetate	30	27	27	30
Sodium gluconate	0	23	23	0

Source: Modified from Gulliksson H. Platelet additive solutions: Current status. Immunohematology 2007;23(1):14–17.
[a]Currently, the only platelet additive solution FDA approved for use in the United States.
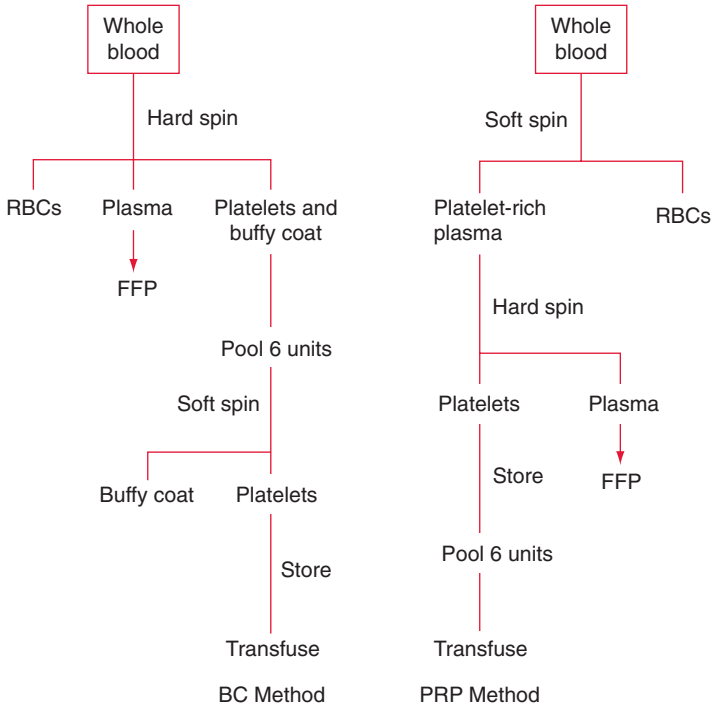
Figure 5.3 Comparison of platelet-rich plasma (PRP) and buffy coat (BC) methods of platelet preparation.

volume of the whole-blood-derived platelet concentrate, these units usually have a volume of about 50 mL to maintain viability and function during storage.

There are two methods for preparing platelets: the platelet-rich plasma (PRP) method and the buffy coat method (Figure 5.3) [46]. In the United States, platelets are prepared using the PRP method; in Europe and Canada, the buffy coat method is used [46, 47]. The PRP method uses low g-forces ("soft" spin), and the PRP easily separates from the red cells [48]. The PRP is transferred into a satellite bag to separate it from the red cells (Figures 5.2 and 5.3). This must be done within 8 hours after the blood is collected. The PRP is then centrifuged at higher g-forces ("hard" spin) and the platelet-poor plasma is removed, leaving a platelet concentrate and about 50 mL of plasma (Figure 5.3). After the plasma is removed, the platelet concentrate is left undisturbed or, preferably, placed on the platelet storage rotating device for 1 hour to minimize platelet damage and allow for spontaneous resuspension [49]. In the PRP method, the first step is a soft spin and the second step a hard spin (Figure 5.3); because of the soft spin, about 20% of the plasma and 20–30% of the platelets remain with the red cells [46]. Another 5–10% of platelets are lost during the second centrifugation step when the PRP is converted to a platelet concentrate. Thus, the PRP method yields about 60–75% of the original platelets, a red

cell unit containing about 40 mL of plasma, and about 50% or more of the leukocytes in the original unit of whole blood. The disadvantages of this method are the loss of some plasma that could be used for fractionation and the high leukocyte content of the platelets.

When platelets are produced by the buffy coat method, the whole blood is centrifuged at a higher g-force (hard spin) to create a buffy coat that also contains most of the platelets (85%) and leukocytes [46, 50]. Because the whole blood centrifugation step involves higher g-forces, the red cells are more tightly packed and more plasma is obtained along with the buffy coat. However, to obtain most of the buffy coat, it is necessary to remove some of the red cells, and so there is a loss of about 20–25 mL of red cells [46]. To obtain a platelet concentrate, the buffy coat is centrifuged using low g-forces, and the platelets are separated from the leukocytes and red cells. Thus, in this method, the first step is a hard spin and the second step a soft spin—the opposite of the PRP method. It is thought that the use of the soft spin in the second centrifugation may result in platelets that function better than those obtained by the PRP method, in which the second centrifugation is a hard spin when there is less whole blood to "cushion" the platelets [50, 51]. The effectiveness of the second centrifugation step is improved if several units of buffy coat are pooled, usually in groups of six [50]. When units of buffy coat are pooled for the second centrifugation, they may be suspended in an artificial platelet preservation (platelet additive) solution that improves the separation and the quality of platelets during storage [52]. Also during the second centrifugation step, the platelets are passed through a filter as they are separated, thus removing most of the leukocytes and producing a leukocyte-depleted platelet component.

Storage conditions and duration

Platelets stored at 20–24°C maintain functional effectiveness for several days [53–57]. Subsequent studies have established that many variables affect the quality of platelets during storage. In addition to temperature, these other variables include the anticoagulant–preservative solution, storage container, type of agitation, anticoagulant, and volume of plasma [58–61]. Gentle horizontal agitation is preferable to end-over-end agitation [61]. If continuous agitation is interrupted, platelets stored for up to 5 days maintain appropriate in vitro characteristics for up to 24 hours of interruption of agitation [62]. The composition, surface area, and size of the storage container influence the ability for carbon dioxide to diffuse out and oxygen to enter the platelet concentrate, and storage containers specifically designed to optimize platelet quality are now used routinely [63, 64].

Maintenance of the pH above 6.0 is the crucial factor indicating satisfactory platelet preservation. This combination of storage container, agitation, preservative solution, temperature, and the use of about 50 mL of plasma provide satisfactory preservation of platelets for up to 7 days [63, 64]. However, several instances of bacterial contamination of platelet concentrates stored for this period were reported [65, 66], and the storage

time was reduced to the 5 days currently used [11]. The problem of bacterial contamination still exists (see Chapter 14). Several platelet concentrates are usually pooled to provide an adequate dose for most patients (see Chapter 11). For some patients, the volume of plasma in the final pooled component is too large, and plasma must be removed prior to transfusion. This involves another centrifugation step after the platelets have been pooled that causes a loss of 15% to as much as 55% of the platelets [67,68].

The leukocyte content of the platelet concentrates is an important issue (see Chapters 11, 12, and 14). The conditions used to centrifuge whole blood influence the leukocyte content of the platelet concentrate, but most platelet concentrates contain 10^8 or more leukocytes. Filters are available that remove most of the leukocytes in the platelet concentrate. The filters can be used at the bedside, or preferably before the platelets are stored. Presently most platelets are leukodepleted.

Leukodepletion of platelets

Filters are available for leukodepletion of platelets as well as red cells. This is necessary if it is hoped to prevent alloimmunization or disease transmission in patients receiving platelet transfusion [35]. The platelet filters result in a loss of about 20–25% of the platelets and have a rate of failure in achieving fewer than 5×10^5 leukocytes of about 5–7% [35].

Granulocytes

Granulocytes for transfusion are prepared by cytapheresis (see Chapter 7). Some investigators have prepared granulocytes from fresh whole blood by sedimentation with hydroxyethyl starch. Doses of 0.25×10^9 are reported from units sedimented with hydroxyethyl starch, and this is below the $1–3 \times 10^9$ desired for transfusion even to a neonate [69]. The possibility of obtaining granulocytes from units of whole blood is usually raised in a crisis; the blood bank often does not have procedures to prepare the cells, and it is not possible logistically to test the blood for transmissible disease. Thus, preparation of granulocytes from units of fresh whole blood is not a recommended approach.

5.2 Irradiation of blood components

The techniques and clinical indications for irradiating blood components are described in Chapter 11.

5.3 Hematopoietic stem and progenitor cells

Hematopoietic stem cells are being obtained from bone marrow, peripheral blood, and cord blood. Collection of marrow and umbilical cord blood is described in Chapter 18 and peripheral blood stem cells in Chapter 7. Stem cells from these different sources are undergoing an increasing variety of cellular engineering methods that produce new blood components with exciting therapeutic potential.

5.4 Plasma derivatives

General

Procedures for the fractionation of plasma were developed during the 1940s in response to World War II (see Chapter 1). A large pool of plasma, often up to 10,000 L or 50,000 donor units, is processed using cold ethanol fractionation. In cold ethanol, different plasma proteins have different solubilities, which allows their separation. This large-scale separation and manufacturing process results in the isolation of several proteins from plasma that are prepared for therapeutic use. These are called plasma derivatives (Table 5.4). The major derivatives have been albumin, immune serum, immune globulin, and coagulation factor VIII concentrate. Until the late 1980s, techniques were not available to sterilize some blood derivatives after manufacture. Thus, because of the large number of units of donor plasma in each pool, the chance of contamination of the pool with viruses (i.e., hepatitis and HIV) was high and the risk of disease transmission from these nonsterilized blood derivatives was high (see Chapter 15). This risk was accentuated because much of the plasma that serves as the raw material for the manufacture of blood derivatives was obtained from paid donors, a group known to provide blood with an increased likelihood of transmitting disease [70, 71]. Initially, only albumin and immune globulin carried no risk of disease transmission; albumin because it was sterilized by heating and immune globulin because none of the known infectious agents was contained in that fraction prepared from the plasma. Because of the recognition of the high risk of disease transmission by coagulation factor concentrates, methods were developed to sterilize them [72, 73].

New concerns have arisen about the possible transfusion transmission of the agent responsible for variant Creutzfeldt–Jakob disease (vCJD) because this infectivity is not inactivated by most conventional methods. Fortunately, it appears that the prions associated with vCJD do not partition with the therapeutic proteins during plasma fractionation [74, 75].

Coagulation factor concentrates

Although these concentrates were known to transmit hepatitis since they first became available, the risk has been reduced over the years by improvements to the donor history, the addition of laboratory tests for transmissible agents, and the introduction in the mid-1980s of methods to treat the concentrates to separate and inactivate viruses [72, 73, 76]. The major methods of viral inactivation for plasma-derived concentrates are (a) dry heating, in which the sealed final vial is heated between 80°C and 100°C, (b) pasteurization, in which the concentrate is heated to 60°C while still in solution before lyophilization, (c) vapor heating, in which the lyophilized powder is exposed to steam before bottling, and (d) solvent–detergent (SD) treatment, in which the organic solvent tri-*n*-butyl-phosphate (TNBP) and the detergent Tween 80 or Triton X100 are added at intermediate processing steps. At present, the SD method is

most commonly used. The pasteurization and vapor heating methods result in substantial loss of factor VIII activity [76, 77].

Each of these methods uses a different strategy of viral inactivation. There are differing amounts of data about the effectiveness of these viral inactivation methods, since not all of their products have been subjected to randomized controlled trials. In general, it appears that the methods are effective in inactivating virus with a lipid envelope, but infections with nonlipid envelope viruses such as parvovirus B19 [78] and hepatitis A [79] have been reported.

The first recombinant-produced coagulation factor VIII concentrates became available in late 1992 and 1993 [80, 81]. It appears that these products transmit no diseases. The factor VIII is produced in murine cell lines, and both fetal calf serum and murine monoclonal antibodies are used in the production process. The products are subjected to viral inactivation steps, even though there should be no way that human viruses should contaminate the products.

Experience with these recombinant products is very encouraging. Although factor VIII is a very antigenic protein, it does not appear that recombinant factor VIII is more likely than plasma-derived factor VIII to cause development of factor VIII inhibitors.

Coagulation factor VIII concentrates produced by recombinant DNA techniques are more expensive than those produced from plasma [82]. Despite this, the high-purity (high-cost) plasma-derived and the recombinant products are the most widely used.

Factor IX concentrates are also free of transmission of most viruses since 1991. Factor IX concentrates vary in purity and most contain additional coagulation factor (Table 5.11) [83]. The less pure concentrates contain other coagulation factors and cause some degree of hypercoagulability

Table 5.11 Coagulation factor IX products currently available.

Category	Product	Manufacturer	Factor IX IU	Other ingredients units per IU of factor IX			
				Factor II	Factor VII	Factor X	Heparin
Purified factor IX concentrates	Bebulin VH	Baxter	500–700 IU	x	Minimal	x	≤0.15
	Profilnine	Grifols	400, 1000, 1500	≤1.5	≤0.035	≤1.0	–
	Alphanine	Grifols	150	≤0.05	≤0.04	≤0.05	≤0.04
	Berinin P	Behring	300, 600, 1200	x	x	x	–
	Mononine	Behring	500, 1000	≤0.0025	≤0.0025	≤0.0025	–
	Octanine	Octapharma	250, 500, 1000	x	x	x	x
Recombinant factor IX concentrates	Benefix	Wyeth	200	–	–	–	–
Prothrombin complex concentrates	Beriplex	Behring	250, 500	0.8–1.92	0.4–1.0	0.88–2.4	x
	Cofact	Sanquin	250, 500	0.56–1.4	0.28–0.8	0.56–1.4	–
	Octaplex	Octapharma	500	0.44–1.5	0.36–0.96	0.72–1.2	0.2–0.5

Note: 1 IU = amount of factor IX activity present in 1 mL pooled normal human plasma.
x indicates amount of not specified substance present.
– indicates element is absent.

[84]. Activated factor VII, fibrinogen, prothrombin complex concentrates, von Willebrand factor concentrate, and fibrin sealant are discussed in Chapter 11.

Fibrinogen

A virally inactivated fibrinogen concentrate prepared from human plasma is now available commercially and is approved by the Food and Drug Administration (FDA) for the treatment of acute bleeding episodes in patients with congenital fibrinogen deficiency [85–87]. The concentrate is recommended for use in situations when the fibrinogen level is less than 100 mg/dL. While fibrinogen concentrate is approved only for use in congenital fibrin deficiency [86], small studies of its use in patients with low fibrinogen levels and massive bleeding from obstetric complications, cardiovascular surgery, intra abdominal surgery, trauma [85], and an aortic ascending aorta replacement [87] have demonstrated substantially reduced bleeding.

Immune serum globulins

Immune serum globulin (Ig or gamma globulin) prepared by the traditional plasma fractionation technique has been very effective in preventing bacterial infections in patients with agammaglobulinemia and in preventing certain viral infections in immunologically normal persons. This immune globulin is administered intramuscularly because it contains aggregated or oligomeric molecules of Ig, which, when injected intravenously, activate complement, resulting in severe reactions [88]. The limitations of IM-IG are dose limitations, painful injections because of the volume required, and difficulty maintaining plasma levels of IgG. Immune globulin suitable for intravenous administration is also available. This is prepared from the plasma of normal donors and thus can be expected to have an antibody content reflective of normal healthy individuals in a large population. There are some differences among different products in the IgA content, the relative proportions of IgG subclasses, and in vitro activity against some viruses. The differences in IgA content are clinically important, as brands that contain much IgA may cause a reaction if given to an IgA-deficient patient with anti-IgA. The importance of the other differences among the brands has not been established. The intravenous half-life of the intravenous immunoglobulin is 21–25 days, which is similar to native IgG.

Intravenous immune globulin (IVIG) is approved for use by the US FDA for treatment of individuals with impaired humoral immunity, specifically for primary (congenital) immune deficiency and for (idiopathic) autoimmune thrombocytopenia [89]. The availability of intravenous immunoglobulin makes it possible to maintain the serum IgG level near normal in immunodeficient patients. The amount required varies with the size of the patient and the indication. Usually 100–200 mg/kg per month is used as a starting dose for patients with primary immunodeficiencies.

Administration of IVIG in autoimmune situations may seem odd. The mechanism of action is thought to be macrophage Fc receptor blockage by immune complexes formed between the IVIG and native antibodies. IVIG is effective for patients with autoimmune thrombocytopenia. Specific IV anti-Rh(D) is used in Rh-positive patients with autoimmune thrombocytopenia [89, 90]. This is thought to cause immune complexes with anti-Rh and the patient's Rh-positive red cells, resulting in Fc receptor blockade. Larger doses are usually used for patients with autoimmune thrombocytopenic purpura compared with immune deficiency. Intravenous immunoglobulin is now used in other immune deficiency or autoimmune states (see Chapter 11)

Adverse reactions to intravenous immunoglobulin occur with 2–10% of injections [89]. These are local, such as erythremia, pain, phlebitis, or eczema. Systemic symptoms include fever, chills, myalgias, back pain, nausea, and vomiting. Some reactions in some patients are dose related and can be reduced or eliminated by slowing the rate of infusion. The nature and frequency of adverse reactions may differ among the different products, but this is not clear and is beyond the scope of this chapter.

Since intravenous immunoglobulin is made from large pools of human plasma, it contains a variety of antibodies, including those against blood groups and possibly anti-HBs, anti-HBc, anti-cytomegalovirus, etc. [89]. Donor screening should eliminate some of these (i.e., anti-HIV), but patients may have transiently positive tests for certain antibodies, especially ABO, that are passively acquired from the intravenous immunoglobulin [91] and some patients may develop a positive direct antiglobulin test. Transient hemolysis has been reported in patients with autoimmune thrombocytopenic purpura and others being treated with IVIG (see Chapter 14). Thus, although hemolysis is unusual, a large proportion of patients receiving IVIG will develop circulating or cell-bound blood group antibodies. This should be considered if unexplained hemolysis occurs in patients being treated with IVIG.

5.5 Pathogen-inactivated blood components

The approach to blood safety during the last 40 years has been very effective but is nearing the end of its effectiveness [92–95]. Addition of new tests and/or screening measures erodes the donor base unnecessarily and is reactive, allowing patients to be harmed before preventive steps are implement. Several pathogen-inactivated plasma products and two pathogen inactivated platelet products are widely used outside the United States but are not FDA licensed for domestic use. This has led to considerable frustration with lack of availability of these products in the United Sates, since it does not appear that the paradigm of increasing donor question and testing can be effective and efficient in the future. Thus, treatment of blood products to inactivate contaminating infectious agents is a new paradigm for the future. Several technologies are now in use.

Solvent–detergent plasma

Treatment of fresh plasma with a combination of solvent TNBP and the detergent Triton X100 inactivates lipid envelope viruses while retaining most coagulation factor activity. The process must be done on a large scale, and plasma from about 2500 donors is pooled for the SD process. The product has little, if any, risk of transmitting lipid envelope viruses such as HIV, HCV, and HBV but can transmit nonlipid envelope viruses such as parvovirus [96]. Reports of thrombosis in thrombotic thrombocytopenic purpura (TTP) patients undergoing plasma exchange with SD plasma [97] and deaths in patients receiving SD plasma while undergoing liver transplantation led to withdrawal of that product from the market, and it is no longer available. It is postulated that these thrombotic complications were due to decreased protein S and plasmin inhibitor activity in SD plasma [97]. A different SD plasma, Octaplas (Octapharma; Vienna, Austria) (Table 5.12), has higher, although not normal, levels of protein S and plasmin inhibitor [98] and has not been associated with thrombotic events. This form of SD-treated plasma is in rather wide use in Europe [99, 100] but not yet in the United States.

Fresh frozen plasma

Three pathogen-inactivated FFP products are in use in Europe. Methylene blue can be added to plasma and subsequent exposure to visible light inactivates most viruses and bacteria [101, 102]. The plasma can then be frozen as an FFP product. Two other pathogen inactivation methods are used for both plasma and platelets [92–95]. One uses a psoralen compound and ultraviolet (UV)-B light [103], while the other uses riboflavin and UV-B light [104]. A psoralen compound followed by UV

Table 5.12 Coagulation factor and inhibitor levels in 12 lots of Octaplas.

Measure	Reference range	Octaplas (n = 12)[a]
PT (s)	12.5–16.1	13.3 (12.9–13.8)
aPTT (s)	28–40	35 (34–37)
Fibrinogen (g/L)	1.45–3.85	2.5 (2.4–2.6)
Prothrombin	65–154	83 (79–86)
FV (U/100 mL)	54–145	78 (75–84)
FVII (U/100 mL)	62–165	108 (90–117)
FX (U/100 mL)	68–148	78 (75–80)
FVIIa (mU/mL)	25–170	166 (134–209)
Protein C activity (U/100 mL)	58–164	85 (81–87)
Protein S activity (U/100 mL)	56–168	64 (55–71)
PI (U/100 mL)	72–132	23 (20–27)
Plasminogen (U/100 mL)	68–144	96 (92–101)
Citrate (mM)		17.5 (14.2–20.9)

Source: Adapted from Solheim BG, Hellstern P. Composition, efficacy, and safety of S/D-treated plasma. Transfusion 2003; 43:1176–1178.
Note: PT, prothrombin time; aPTT, activated partial thromboplastin time.
[a]Data are reported as mean (range).

light results in intercalation into DNA or RNA with cross-links. Riboflavin damages DNA upon exposure to UV light. Both methods prevent nucleic acid replication. Thus, contaminating pathogens are inactivated, but platelets are not damaged. Extensive toxicity, mutagenicity, and pharmacologic studies have given satisfactory results. The psoralen product has satisfactory coagulation factor levels and provides post-transfusion increases in coagulation factors similar to ordinary FFP [103]. The riboflavin-treated FFP also has satisfactory levels of coagulation factors [104]. Because these products are relatively new, there is little clinical experience reported, but the psoralen product is effective in patients with bleeding due to liver disease [105] and for replacement in patients with thrombotic thrombocytopenic purpura [106].

Platelets

Two of the methods used for pathogen inactivation of FFP are also being used to treat platelets [92–95, 107]. Initial studies in healthy research subjects and studies in thrombocytopenic patients indicate satisfactory platelet function for both the psoralin and riboflavin methods [108–110]. Successful clinical trials in Europe using psoralin-treated platelets prepared by the buffy coat method [111] and in the United States using apheresis platelets [112] have been reported [113] and those platelets are widely used [114]. Riboflavin-treated platelets also appear to be clinically effective [113, 115].

UV-C light is also being developed as for pathogen inactivation of platelet concentrates [116–118].

Red cells

Two different approaches are under development for inactivation of transfusion transmissible pathogens in red blood cell components. These involve riboflavin [119] and an alkylating agent [120]. The methods involve selective damage to nucleic acid strands, thus inactivating contaminating pathogens while sparing red cells. The methods are effective against most common bacteria, viruses, and protozoa that would be of concern in blood transfusion. The methods are at early stages of development, but early in vivo studies in humans are said to show satisfactory results, although data were not published as of this writing.

Inactivation of viruses and bacteria in cellular components, a strategy almost unthinkable a decade ago, is also showing exciting promise. If pathogen inactivation is broadly effective, there will certainly be a major impact on the blood supply system and the nature of blood centers producing these components.

5.6 Universal red cells

Two approaches have been attempted to convert A or B red cells to type O. If such a process became practical and widely adopted, it could have a huge impact on blood banking by eliminating most inventory management issues and making more blood available by eliminating outdating of type A

and B units [121]. Development of these technologies has been difficult and neither are near clinical use.

Enzymatic cleavage of ABO and Rh antigen
Cleavage of ABO and Rh antigen

Strategies have been devised for the enzymatic cleavage of the sugars that confer A and B specificities [121–123]. The enzymes for this cleavage have been cloned and are available on a scale sufficient to allow for the production of clinical doses of red cells from which the A and B antigens have been removed. Thus far, most of the experience involves successful conversion of group B to group O [122]. It is not clear whether A to O conversion will be possible, and no clinical data have been reported despite completion of a phase clinical trial in 2007 [123].

Masking ABO antigens

A different approach to altering the red blood cell membrane to convert group A or B red cells into group O red cells is to mask the antigens to produce "stealth" red cells. Polyethylene glycol has been used to covalently bond to red cells to mask blood group antigens such as ABO, Rh, Kell, and Kidd [124]. Small studies in animals suggest that there is little in vitro damage to the red cells and that they have a normal survival, although such studies have not yet been carried out in humans. It is not clear that development of this process will continue.

5.7 Blood substitutes

The functions of blood can be grouped generally as maintenance of intravascular volume, delivery of oxygen to tissues, provision of coagulation factors, provision of some defense mechanisms, and transportation of metabolic waste products. Considerable effort has been made to develop blood substitutes or artificial blood, but these products deal only with the oxygen-delivery function. Thus, more appropriate terms are hemoglobin or red cell substitutes [125].

The ideal acellular red cell substitute would not require crossmatching or blood typing, could be stored preferably at room temperature for a long period, have a reasonable intravascular life span and thereafter be excreted promptly, and be free of toxicity or disease transmission. Two approaches have been used: perfluorocarbons, compounds in which oxygen is highly soluble, and free hemoglobin solutions using either human or animal hemoglobin [126]. Hemoglobin chemically binds oxygen, whereas perfluorocarbons have a carbon backbone with fluorine substitutions that have solubility for oxygen 20 times greater than water. The physiologic benefit of this high solubility for oxygen has been demonstrated dramatically by the survival of mice completely immersed in a solution of well-oxygenated perfluorocarbons.

Hemoglobin can be prepared in solution by lysis of red cells. If the remaining cell stroma is removed, the stroma-free hemoglobin is nonantigenic. However, stroma-free hemoglobin in solution has a short

intravascular life span and has a low P_{50} (the point at which 50% is saturated). Thus, research has focused on modifying the structure of the hemoglobin molecule (cross-linking or polymerization) or binding hemoglobin to other molecules to overcome these two problems [126]. Outdated human red cells, bovine hemoglobulin, and recombinant DNA-produced hemoglobin have been used as sources of hemoglobin. The potential difficulties with hemoglobin-based oxygen carriers are rapid clearance of the hemoglobin, hypertensive effects, change in the oxygen dissociation curve, hemoglobin metabolites, immunogenicity, and bacterial sepsis [126].

Four products are or have undergone clinical trials: Polyheme (Northfield Laboratories, Evanstone, IL), HemAssist (Baxter Healthcare Corporation, Round Lake, IL), Hemopure (Biopure Corporation, Cambridge, MA), and Hemolink (Hemosol, Mississauga, Ontario). Development of HemAssist has been discontinued after randomized trials demonstrated safety problems [127, 128]. Hemopure was used successfully in a patient with severe autoimmune hemolytic anemia [129] and in a sickle cell disease patient with acute chest syndrome who refused blood transfusion [130]. However, clinical trials of these products seem to have come to a stop [131, 132] and the long-awaited "blood substitute" is not close to reality.

In a careful study, 8 severely anemic patients (hemoglobin levels of 1.2–4.5 g/dL) who refused a blood transfusion received the perfluorocarbon product were compared with 15 who did not [133]. The amount of oxygen delivered by the perfluorocarbon was not clinically significant, and the patients did not benefit. The major observation in this study was the ability of all the patients to tolerate remarkably low hemoglobin levels and the lack of the need for increased arterial oxygen content in the 15 control patients who had hemoglobin levels of approximately 7 g/dL. Fluosol products are neither available nor undergoing clinical trial.

Potential clinical uses and impact of hemoglobin substitutes

If a hemoglobin-based oxygen carrier was developed, it is not likely that it will supplement most red cell transfusions. The substitutes might be used for immediate restoration of oxygen delivery such as in trauma, or in other urgent situations involving massive blood loss where red cells are not available quickly, but the short intravascular half-life of these substitutes makes them impractical for long-term red cell replacement (for instance, in chronically anemic patients). Since blood typing and crossmatching would not be necessary, the substitutes might be carried in emergency vehicles, stocked in emergency departments, or used by the military or civilians in situations where access to blood is limited. Other potential uses of hemoglobin substitutes include organ perfusion and preservation prior to transplantation, and improving oxygen delivery to tissues that have an impaired blood supply. Unfortunately, it does not appear that a hemoglobin-based blood substitute will be available soon.

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6 Autologous Blood Donation and Transfusion

Autologous blood is widely believed to be the safest blood [1] and, as a result, interest in and the use of autologous blood increased dramatically with the onset of the HIV epidemic. The most common form of autologous blood is that deposited in anticipation of elective surgery, also called preoperative autologous blood donation (PABD). Other forms of autologous blood are perioperative or acute normovolemic hemodilution (ANH), intraoperative salvage of shed blood, and postoperative salvage of shed blood.

6.1 Strategies to reduce or avoid allogeneic transfusion

Concerns about blood safety have also increased interest in other methods to avoid the need for transfusion or the development of "limited" donor programs. Several strategies can be used in a combined approach to minimize the use of allogeneic blood (Table 6.1) [2–4]. A wonderful, comprehensive program for minimizing the necessity of transfusion of allogeneic blood is provided by one of the pioneers of cardiovascular surgery, Dr. Denton Cooley [3]. This program involves preoperative considerations such as review of the medical history to identify factors that may predispose patients to excessive blood loss, review of medications that might increase blood loss, use of erythropoietin (EPO), and PABD, as well as intraoperative considerations such as the use of ANH, close attention to heparin levels, use of antifibrinolytic agents, use of hypothermia, attention to hemostasis, and intraoperative and postoperative salvage of shed blood (Table 6.2).

The indications for transfusion are a major factor in determining blood utilization. During the past decade, patients' hemoglobin levels or platelet counts have been allowed to fall much lower before transfusion, and the use of plasma has been curtailed (see Chapters 11 and 12). These changes in the indications for transfusion have resulted in fewer transfusions and thus fewer donor exposures. Pharmacologic agents may be used to reduce blood loss [2], and are more careful use of anticoagulants and use of protease inhibitors such as epsilon aminocaproic acid in fibrinolytic situations is helpful. A different approach using pharmacologic agents involves stimulation of hematopoiesis. This can be done for red cells using

Table 6.1 Strategies to reduce or avoid allogeneic transfusion.

Change indications for transfusion
Use special surgical and anesthetic techniques
Use pharmacologic agents to:
   Reduce blood loss
   Stimulate marrow
   Increase capability for autologous donations
Use limited-donor programs
Use single-donor components

EPO either to enhance red cell production in the patient in anticipation of blood loss or to increase the number of autologous units that can be donated. Another strategy that can reduce but not eliminate allogeneic donor exposure is the use of single-donor components when possible. The most common example of this is the use of single-donor platelets obtained by apheresis instead of pooling several units obtained from whole blood to provide one therapeutic dose. Some blood centers make available fresh frozen plasma in large-volume units, thus reducing by about half the number of donor exposures from plasma transfusions. Finally, limited-donor programs are operated by some centers. This is usually practical only for pediatric patients. While all of these strategies are available, it would be unusual to find them all used extensively in one center. Each hospital has its own unique mix of activities designed around these specific steps, but the central theme that pervades transfusion practice today is the more conservative use of blood transfusion and structuring the provision of blood components to take into account the public's concern about transfusion-transmitted diseases.

6.2 Trends in the collection and transfusion of autologous blood

Between 1989 and 1992, there was a 70% increase in autologous blood collections, reaching a maximum of 1,117,000 units in 1992 [5]. However, by 2006, autologous blood collections were only 335,000 or 2.1% of the national blood supply, which was a 27% decline between 2005 and 2007 [6]. This is far short of predictions that autologous blood could provide 20% of all blood used and is unlikely that such extensive use of autologous blood will ever occur.

6.3 Preoperative autologous blood donation

Autologous donor blood

An individual may donate blood for his or her own use if the need for blood can be anticipated and a donation plan developed. Most commonly, this occurs with elective surgery. Surprisingly, the major motivation for

Table 6.2 Blood management considerations for cardiovascular surgery patients.

	Consideration	Comments
Preoperative	Antithrombolytic drugs	Whenever possible, discontinue several days before surgery
	Epoetin alfa	Appropriate for patients at high risk of bleeding complications
	Iron supplementation	Appropriate for patients with low Hb levels or clinical or laboratory signs of anemia
	Preoperative autologous donation of blood	Limited by cost, logistics, and time; recommended only for patients at high risk of bleeding
Intraoperative	Hemodilution	Extreme hemodilution (HCT < 20%) may adversely affect platelet function
	Heparinization	Adequate anticoagulation can usually be achieved with a loading dose of 3 mg/kg
	Aprotinin	Reduces blood loss and transfusion requirements; most useful in patients at high risk of bleeding
	Aminocaproic acid	Antiplasminogenic effects
	Desmopressin	Useful in some patients with coagulopathies such as uremic thrombopathy, platelet defects, von Willebrand's disease
	Hypothermia	Moderate levels (22–28°C) should be used; expeditious rewarming reverses adverse effects
	Topical hemostatic agents	Used to create drier operative field and reduce blood loss
	Blood salvage and autotransfusion	Although considered fairly standard practice unwashed blood collected without systemic anticoagulation could increase clotting products and activated clotting factor proteins
Postoperative	Shed mediastinal blood salvage	Can reduce the need for allogeneic RBC transfusion by 50%; generally ineffective if the volume to be reinfused is < 400 mL or 4 h
	Allogeneic transfusion	Should be used only when absolutely necessary
	RBC transfusion	May be needed when Hb < 8 g/dL
	Platelet therapy	Consider when platelet level < 50,000 cells/mm^3
	Fresh frozen plasma	May be needed when PT > 15 s, PTT > 40 s, or postoperative chest tube output > 300 mL/h for 2 h or > 900 mL total in 3 h
	Hypertension	Must be controlled to avoid potential for increased bleeding; plasma expanders can cause or exacerbate hypertension

Source: Cooley DA. Conservation of blood during cardiovascular surgery. Am J Surg 1995; 170:53S–59S.
Hb, hemoglobin; HCT, hematocrit; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cell.

Table 6.3 Autologous blood collection techniques in selected surgical procedures.

Surgical Procedure	Autologous blood collection technique[a]			
	PABD	IBS	PBS	ANH
Coronary artery bypass graft	+	+	+	+
Major vascular surgery	+	+	−	+
Primary hip replacement	+	+	+	+
Revision hip replacement	+	+	+	+
Total knee replacement	+	−	+	−
Major spine surgery with instrumentation	+	+	+	+
Selected neurologic procedures(e.g., resection of arteriovenous formation)	+	+	−	+
Hepatic resections	+	+	−	+
Radical prostatectomy	+	+	−	+
Cervical spine fusion	−	−	−	−
Intervertebral discectomy	−	−	−	−
Mastectomy	−	−	−	−
Hysterectomy	−	−	−	−
Reduction mammoplasty	−	−	−	−
Cholecystectomy	−	−	−	−
Tonsillectomy	−	−	−	−
Vaginal and cesarean deliveries	−	−	−	−
Transurethral resection of the prostate	−	−	−	−

Source: National Heart, Lung, and Blood Institute Expert Panel on the Use of Autologous Blood. Transfusion alert: use of autologous blood. Transfusion 1995; 35:703–711.
ANH, acute normovolemic hemodilution; IBS, intraoperative blood salvage; PABD, preoperative autologous blood donation; PBS, postoperative blood salvage.
[a]+Indicates use of the technique is considered appropriate; − indicates use of the technique is considered inappropriate.

autologous blood donation is the physician's recommendation rather than the patient's fear of transfusion-transmitted infection [7]. It is important to thoughtfully plan the autologous blood donation schedule so that blood is collected only for procedures for which there is substantial likelihood that the blood will be used (Table 6.3) [8]. Without this type of planning, there is a very high rate of wastage of autologous blood, and the costs become quite high. In general, autologous blood is recommended only for procedures for which blood would be crossmatched and that involve at least a 10% chance of blood use. Examples are major orthopedic procedures, radical prostatectomy, vascular surgery, and open-heart surgery [9–11]. Examples of situations in which autologous donation is not recommended include cholecystectomy, herniorrhaphy, and normal delivery. It is essential to define the specific situations in which autologous blood is indicated and then define the amount of blood desired for each of those situations. The hospital's standard surgical blood order system can be used to estimate the amount of blood likely to be used, and a plan for autologous donation can be developed based on that schedule [12, 13]. An

excellent review of autologous blood utilization shows relationships among estimated blood loss, hematocrit changes during hospitalization, cost-effectiveness of autologous blood, and programs for collecting the blood [14]. An example of strategies combining all of the different autologous blood collection and transfusion methods is illustrated in Table 6.2.

If patient candidates for autologous blood donation meet the usual Food and Drug Administration (FDA) criteria for blood donation, their blood may be "crossed over"—that is, used for other patients—if the original autologous donor has no need for the blood. If the autologous donor does not meet the FDA criteria for blood donation, the blood must be specially labeled, segregated during storage, and discarded if not used by that specific patient. There is no general agreement about the desirability of the practice of crossing over autologous units. One study showed similar rates of transmissible disease markers in autologous and allogeneic donors [15], while another reported increased rates among autologous donors [16]. Definitive data are not available. Because the blood bank medical director must approve "crossing over" each unit on a case-by-case basis, most blood banks do not cross over autologous blood to patients other than the donor-patient. Since most autologous (PABD) donors do not meet FDA criteria for allogeneic donation, their blood cannot be crossed over to others and as a result there is a high wastage rate. This poor utilization rate was attributed to donation for procedures with a low probability of use, donation beyond the expected need, lack of clear criteria for transfusing autologous blood, and hesitancy to use suitable autologous blood as part of the general community blood supply [5,6]. Thus, although use of autologous blood is popular and is seen by many patients as a valuable way to reduce the possibility of disease transmission, its use in an operationally efficient and cost-effective way are not well developed.

Occasionally a healthy person wishes to donate blood for long-term storage for himself or herself in the event of a future unforeseen need. This is not recommended because of the low possibility that the blood will be available where and when needed and the high cost of the long-term storage. If the individual has a very rare blood type, it may be desirable to collect blood and freeze it for later use by the donor or for others with similar rare red cell phenotypes.

In summary, experience with PABD has shown that (a) donor-patients often begin surgery with a lower hematocrit (hemodiluted) and thus require more transfusions or are discharged from the hospital with a lower hematocrit, (b) it is suitable for a limited number of patients, (c) nationally it replaces only about 2% of all red cells at best, and (d) it is not cost-effective. Despite this experience, PABD should be an option patients can choose in consultation with their physician.

Medical requirements and evaluation for autologous blood donation

The medical history for autologous donation is the same as for allogeneic donation except that, since the donor is actually a patient, additional

Table 6.4 Cardiovascular conditions proposed as contraindications to autologous blood donation.

Idiopathic subaortic stenosis
Aortic stenosis
Left main coronary artery disease
Unstable angina
Cardiac failure
Recent myocardial infarction
Ventricular arrhythmia
Atrioventricular block
Symptoms of disease on the day of donation

Source: Yomtovian R. Practical aspects of preoperative autologous transfusion. Am J Clin Pathol 1997; 107(Suppl 1):S28–S35.

emphasis should be placed on questions about medications and medical conditions or illnesses. There are no age or weight restrictions for autologous donation. PABD is generally safe for older donors [17, 18]. One of the types of severe reactions in allogeneic donors who are in good health is cardiovascular events [18], and deaths have occurred following blood donation by individuals who met all the FDA criteria for blood donation [19]. Therefore, there is some difference of opinion regarding the safety of donation by patients with cardiovascular risk factors. Several specific cardiovascular conditions have been proposed as contraindications for autologous blood donation [13] (Table 6.4).

Pregnant women may donate, although this is not recommended routinely, since these patients rarely require transfusion and often are iron deficient [8, 20]. Donors with known or suspected bacteremia should not donate autologous blood because of the possibility of transfusion of contaminated blood (see Chapter 15). Examples of such situations are patients with ulcers that might be associated with bacteremia or patients with recent gastroenteritis that might be due to *Yersinia enterocolitica*. The autologous donor's hemoglobin may be lower (11 g/dL) than that required for allogeneic donors (12.5 g/dL), and autologous donors may donate as often as every 72 hours up to 72 hours prior to the planned surgery. Usually it is only possible to obtain 2–4 units of blood before the hemoglobin falls below 11 g/dL. The planned donations ideally should begin 4–6 weeks before the anticipated blood use. Patients with a lower initial hematocrit who experience recovery to the baseline hematocrit or above by the time of surgery and who with a longer time between the first autologous blood donation and surgery benefit from PABD. However, patients who do not meet these criteria obtain more benefit from the use of intraoperative blood salvage [21]. Donations closer to surgery than 15 days are not effective because the patient's hemoglobin does not have time to recover [22–24]. Thus, the patient may receive the blood back due to the iatrogenic anemia induced by the blood donations or leave the hospital more anemic than necessary [25]. Oral iron therapy is usually

recommended to facilitate erythropoiesis in response to blood donation. However, many patients experience gastrointestinal side effects that limit the iron therapy.

The final decision on whether to withdraw blood from an autologous donor rests with the medical director of the blood bank. Often consultation between the donor's (patient's) physician and the blood bank physician is necessary to arrive at a wise course of action.

Collection processing and storage of autologous blood

Procedures for the selection of veins for phlebotomy, cleaning the venipuncture site, use of containers and other equipment for blood collection, and the actual collection are the same as procedures used for collection of allogeneic blood (see Chapter 4). If desired, the amount of anticoagulant in the primary container can be reduced by transferring some anticoagulant to a satellite bag. This makes it possible for autologous blood to be collected from children or small adults [26]. All autologous units must be labeled "for autologous use only." Components can be made from autologous units, but if so, each component must be labeled as autologous, and recording systems must ensure that these components are used only for the donor-patient. Usually autologous blood is collected within a few weeks of its intended use and is stored in the liquid state as is done for the ordinary blood supply. The red cells can be frozen for longer term storage if it is desired to allow the donor's red cell mass to replenish itself. However, this adds substantially to the cost of the blood and is rarely done. Another reason to consider freezing the red cells is if the planned use is delayed. Alternatively, the red cells can be returned to the donor-patient, but this is not recommended because transfusion will suppress the donor's red cell production.

Adverse reactions to autologous blood donation

There is some disagreement regarding whether adverse reactions are more common in autologous than allogeneic donors. This comparison is complicated by the fact that autologous donors tend to be older than allogeneic donors and to have medical conditions that would preclude many of them from allogeneic donation. Two reports show no increase in adverse reactions in autologous donors [18, 27]. Autologous donors most likely to experience a reaction with donation are first-time female donors, younger age group, and lower weight and those taking cardiac glycosides, all of which are independent predictors of donor reactions [18, 28, 29]. Elderly autologous donors are least likely to have reactions [18]. Donors who react at their first donation are very likely to react at subsequent donations. In one study, very severe reactions resulting in hospitalization were found to occur 12 times more often in autologous donors than in allogeneic donors and may occur as often as once in 17,000 donations [22]. Thus, although it is not clear whether the overall incidence of adverse reactions is greater in autologous than allogeneic donors, this form of blood donation is not without risk. Autologous donation should be used

only after careful consideration of the particular patient's medical condition and potential transfusion needs.

Laboratory testing of autologous blood

Autologous blood must be typed for ABO and Rh antigens, just as with allogeneic blood. If the blood is to be kept in the institution where it is collected and used only for the autologous donor, no red cell antibody detection or infectious disease testing is required. If the blood is to be shipped to another institution for transfusion, at least the first unit must be tested for transmissible diseases [30]. Subsequent units donated within 30 days need not be tested [30]. If any of the transmissible disease tests are positive, the unit must be labeled with a biohazard label. This is sometimes confusing or disconcerting to physicians and patients. The biohazard label implies to them that the blood is unsafe, but it is an FDA requirement intended to alert health care personnel to the potential hazard presented by the blood, which has a positive test for a transmissible disease.

At the time of admission to the hospital for the planned blood use, a blood specimen should be obtained from the patient and compatibility testing carried out with the autologous unit in order to check for labeling errors. The nature of the compatibility can be established by the hospital but should consist of at least confirming the ABO compatibility between the patient's sample and the unit of blood. It is preferable to use the hospital's routine compatibility testing system for autologous units as well as allogeneic units to minimize the chance of error caused by special handling of the autologous units.

Donation of autologous blood by patients known to be infectious

Patients known to be infectious may still wish for the benefits of autologous transfusion. This issue raises two concerns that medical personnel might accidentally become infected with the blood (via needle puncture, etc.) and that the blood might be inadvertently transfused to someone other than the patient-donor. Ethical principles that have been applied to this situation include the principle of autonomy, whereby the patient has a right to decide what should and should not be done to him or her, and the principle of justice, whereby equity is ensured to all involved or potentially affected by the decision [31–33]. The denial of treatment to any specific group of patients is considered unethical [31], which seems to indicate that it would be unethical to deny the opportunity to donate autologous blood to a patient solely on the basis of HIV or other infectious disease status. A detailed analysis of the risks and benefits indicated that these are in balance only when donation is by a patient with hepatitis C [34]. Any other kinds of infections carry greater risks of harm to others than benefits to the patient. While policy decisions depend on the "interplay of the medical utility of such blood and the public health implications of an accident or error resulting in the inadvertent transmission of virus to an innocent party," [31] each institution must establish its own local policy on this issue and each patient situation assessed.

Use of erythropoietin to increase autologous blood donation

Administration of EPO might be of benefit to potential donors of autologous blood in at least three ways: (1) make it possible to donate more blood; (2) make it possible to donate when the patient might not have been able to do so at all because of anemia; and (3) provide faster recovery of hemoglobin after autologous donation, resulting in a higher hemoglobin at the time of surgery. Administration of EPO to autologous blood donors increases the number of units of blood they can donate [35, 36]; however, EPO administration usually has not been shown to reduce the need for allogeneic donor blood [36, 37]. In one study of orthopedic surgery in rheumatoid arthritis patients, the use of EPO did increase the amount of blood donated preoperatively but also the amount of allogeneic blood the patients received [36]. Another example of a specific situation in which EPO administration was helpful is a patient with anemia of chronic disease who had multiple red cell antibodies and in whom EPO made possible autologous donation for elective surgery [38]. In general, the main advantage of EPO administration may be in patients who are anemic prior to considering autologous donation or who are not candidates for PABD [39]. These studies also established that the increased red cell production that is under way at the time of surgery can reduce the need for allogeneic blood use even in the absence of autologous blood [35, 40].

Utilization of preoperative autologous donated blood

Overall, only about half of PABD blood is used [5, 6]. This wastage contributes to the high cost of autologous blood, but another major issue is whether the donation of autologous blood causes changes in the patient on admission to the hospital that would alter the transfusion therapy. That is, if the patient is admitted with a lower hemoglobin level, he or she may ultimately receive more blood than comparable patients who have not donated autologous blood. This was substantiated in one study in patients who donated autologous blood prior to elective hysterectomy had lower hematocrits upon admission and received more transfusions during their hospitalization [41]. In general, it seems quite likely that patients who have donated autologous blood will have lower hemoglobins at the time of elective surgery. This raises the question of whether the indications for transfusion of autologous units should be the same as for allogeneic units. Because there are risks associated with autologous units, many transfusion medicine physicians have suggested that indications should be the same. Others contend that since the risk–benefit relationship for autologous transfusion is different than for allogeneic transfusion, the indications should be different. There are some data suggesting that those who donate autologous blood have a different transfusion experience from patients who do not. For instance, orthopedic surgery patients who had donated autologous blood received fewer transfusions than nondonors [42, 43]. Thus, although the availability of autologous blood might lead physicians to transfuse it more liberally, some data, such as the previous examples, suggest that physicians might be more conservative when autologous

blood is available. Each hospital must establish its own guidelines regarding the indications for autologous blood. Suggested transfusion audit criteria have been published [44, 45].

Cost-effectiveness

The cost of autologous blood is greater than that of allogeneic blood. Additional time is required for scheduling, for the donor interview, for labeling and making decisions regarding transmissible disease testing, and for record keeping. Other costs of autologous blood are for special shipping if the unit is not collected in the hospital where it will be used and for additional handling and quarantine within the hospital blood bank. Most blood banks apply a surcharge or extra handling fee to cover these additional costs. Ironically, although autologous blood is considered the safest blood for the patient, governmental agencies and health care providers may refuse to pay the extra costs of autologous blood, thus placing a financial disincentive on its use. Because approximately 50% of autologous blood donated preoperatively is not used [5, 6], this results in other additional costs. A study of the cost-effectiveness of preoperative autologous blood [46] demonstrated that the expected health benefit was only approximately 2–4 hours of additional life span at a cost of $235,000 to $23 million per quality-adjusted life year. Separate studies have estimated that the cost per life year saved ranged from $40,000 to more than $1 million when autologous blood was used, depending on the likelihood of transfusion during the surgical procedures [47, 48]. Thus, autologous blood is considerably more costly than allogeneic blood for the benefits received and does not fit within the usual range of medical procedures thought to be cost-effective [25]. However, the widespread use of autologous blood is another example of the lack of impact of cost-effectiveness on the specific practices used within transfusion medicine as illustrated by the cost of $1.5–4.3 million per quality adjusted life year from introduction of nucleic acid testing for HIV and HCV [49].

Complications of transfusion of autologous blood

Although it is widely accepted that autologous blood is the safest blood for a particular patient, complications of autologous transfusion can occur (Table 6.5). These include problems in handling the autologous blood, such

Table 6.5 Complications of autologous transfusion.

Donor-related	Bacterial contamination of unit
Clerical	Allogeneic units used out of sequence when autologous units available
	Autologous units not available when needed
	Autologous unit transfused to wrong patient; hemolysis disease transmission
	Autologous unit allowed to outdate
Mechanical	Hemolysis due to improper collection, handling, or storage of unit
	Hemolysis due to improper transfusion technique

as allowing units to outdate, units being misplaced by the blood bank, or transfusing allogeneic units out of sequence instead of available autologous units. If the donor has bacteremia, the unit may be contaminated and the patient then receives contaminated blood. If the donor has unapparent hemolytic syndrome, hemolysis can occur at the time of transfusion [50]. Rarely blood is improperly handled or the transfusion techniques are not satisfactory (see Chapters 13–15), resulting in the transfusion of hemolyzed blood. These same problems can occur with autologous blood. The most serious problem is transfusing the unit to the wrong patient. In one study of 251,228 autologous units, it was determined that transfusion errors occurred once for every 15,600 units or once in 14,800 patients [51] and the NIH Expert Panel concluded that the likelihood that a unit of autologous blood will be given to the wrong patient is 1 in 30,000–50,000 units [8]. In a questionnaire to American Association of Blood Banks institutions, 1.2% reported erroneously transfusing a unit to the wrong patient [13]. The overall estimate of the risks of autologous transfusion then can be estimated as 1 per 15,000–50,000 units.

6.4 Acute normovolemic hemodilution

As experience has been gained with the successful use of colloid and crystalloid to manage acute blood loss, this approach has been applied in a controlled setting to collect blood from patients for autologous transfusion. This has been called ANH [52, 53]. In acute ANH, whole blood is withdrawn immediately before or after induction of anesthesia. As the blood is removed, blood volume is maintained with infusion of large volumes of crystalloid (3:1 ratio with volume of blood removed) or colloid (1:1 ratio with volume of blood removed), although colloid may be preferable [54]. The amount of blood removed is that projected to reduce the hematocrit level to approximately 28%, although more extreme reductions of hematocrit are sometimes attempted. There are several theoretical advantages of ANH [55] (Table 6.6).

During hemodilution, compensatory mechanisms that maintain oxygen delivery include increased cardiac output [56, 57] and decreased blood viscosity with resulting decreased peripheral vascular resistance, thus enhancing cardiac output decreased peripheral vascular resistance, which is maximal at a hematocrit level of approximately 30% [58], and maximum

Table 6.6 Theoretical advantages of acute normovolemic hemodilution.

- Production of autologous blood
- Availability of fresh blood containing platelets and coagulation factors
- Reduction in the amount of red cell loss during surgery because intraoperative bleeding occurs at a lower hematocrit after blood donation
- Improved hemodynamics and oxygen availability due to the lower hematocrit
- Decreased operating time
- Possibly postoperative improvements in pulmonary, renal, and myocardial function

Table 6.7 Criteria for acute normovolemic hemodilution.

Expected blood loss >1 liter or 20% blood volume
Hemoglobin >12 g/dL
Absence of coronary heart disease
Absence of coagulopathy
Absence of liver disease
Absence of severe hypertension
Absence of severe pulmonary disease
Absence of severe renal disease

Source: Adapted from Stehling L, Zauder HL. Acute normovolemic hemodilution. Transfusion 1991; 31:857–868.

oxygen transport capacity at the hematocrit level of approximately 30% [59]. Thus, it is thought that the patient may have better hemodynamics at the lower hematocrit that results from the blood donation just prior to surgery.

In ANH, the blood is collected from large-bore catheters placed into the central vein or even an artery (usually the radial artery). The blood is collected into standard plastic blood bank containers containing CPD anticoagulant and stored at room temperature, usually right in the operating room [59–61]. It is essential that the personnel collecting the blood be familiar with important steps such as mixing the blood during collection, proper labeling, and storage conditions. Specific procedures should be available, and the staff should be familiar with them. Formulas are available to calculate the volume of blood that can be removed from patients with different starting hematocrits and different weights to achieve a final hematocrit of 30% [61]. Criteria for patient selection should be determined in advance by written protocol (Table 6.7). Usually ANH is reserved for patients in whom the expected blood loss is 1 liter or more or 20% or more of the patient's estimated blood volume.

ANH has been used to provide autologous red cells in orthopedic surgery, major general surgery, liver resection, and cardiovascular surgery [59, 60, 62–72]. There are reports of a 15–90% decrease in the use of allogeneic red cells (multiple studies reviewed) [61]. For total knee arthroplasty, ANH is as effective as preoperative autologous donation reducing allogeneic blood use [73]. This would seem to indicate that ANH is very effective, and from this one would expect that it is widely used. However, there is not universal agreement about the value, safety, and cost-effectiveness of ANH [53]. Despite the references listed above, there are only a few structured studies of ANH and most involve small numbers of patients and few or no controls [53].

Analysis of 42 trials comparing acute normal volemic hemodilution to usual care or another blood conservation method revealed that patients undergoing hemodilution received 1–2 fewer units of allogeneic blood and also they had less bleeding than patients receiving usual care [74]. This large meta analysis supports only modest benefits from the use of ANH

and the authors point out that the safety of the procedure is unproven; therefore, widespread adoption cannot be encouraged without more structured, controlled clinical trials including documentation of adverse events that was done in only one-third of the trials analyzed in this study [74]. Unfortunately, it seems unlikely that large, properly structured, controlled studies will be done. Thus, the data on safety and the effectiveness of ANH in avoiding allogeneic red cell transfusion are not as compelling as it might seem from the references cited. A mathematical model suggests that cell salvage has the potential to offer greater red cell transfusion avoidance than does acute normal volemic dilution [75].

6.5 Intraoperative blood salvage

Development of blood salvage

There are a number of situations in which blood can be collected from the operative site or extracorporeal circuits and returned to the patient. This process is known as intraoperative salvage. With the development of cardiovascular surgery, it became apparent that the blood lost during surgery and in the pump oxygenator could be recovered and returned to the patient. Pump devices were improved, extracorporeal anticoagulation techniques refined, and safety systems such as bubble traps introduced to minimize the likelihood of complications when blood was salvaged from the operative site. This practice became more routine, and it also became apparent that the approach could be used to salvage blood in other types of surgical procedures [76]. At about the same time, it was recognized clinically that blood shed from sources such as the chest was defibrinated and could be returned to the patient after being washed. Further stimulating interest in blood salvage was the dramatic growth in coronary artery bypass surgery during the 1970s and 1980s. Thus, gradually, an incentive developed for manufacturers to develop devices specifically designed for salvage of blood shed during surgery (Table 6.8).

Devices used for intraoperative blood salvage

There are three types of blood salvage devices: [1] canisters, [2] cell processing units, and [3] single-use reservoirs [77, 78]. The canisters use a sterile plastic liner in a rigid container attached to a suction device. Anticoagulant is added as the shed blood is aspirated into the canister. When the container is full, it can be connected to a cell-washing device or removed and taken to the laboratory for washing and concentration of the red cells. These units are less expensive than the other devices and are used when the amount of blood loss is expected to be small. Cell-processing devices are similar to instruments used for apheresis. They are semicontinuous-flow systems in which the blood is aspirated, anticoagulated with heparin or citrate [77], and washed to remove the anticoagulants as well as platelets, plasma, and most debris if present. These devices are more complex and use more expensive software than the

Table 6.8 Intraoperative salvage devices.

Manufacturer	Device	Category
Boehringer	Autovac	Single-use reservoir
Fresenius	Continuous Auto Transfusion System (CATS.)	Cell processing unit
Global blood resources	Hemobag	Ultrafiltration device[a]
Haemonetics	Cell Saver	Cell processing unit
Haemonetics	OrthoPAT	Canister
Haemonetics	CardioPAT	Canister
Medtronic	AutoLog	Canister
Sorin	Electra	Cell processing unit
Sorin	BRAT 2	Cell processing unit
Stryker	Constavac Blood Conservation System	Single-use reservoir

[a]Ultrafiltration devices filter anticoagulated whole blood and return it to the patient.

canisters, and so the semicontinuous devices are used when blood loss is expected to be substantial, usually greater than 1 liter. The single-use reservoir is a simple device that allows salvage of blood with immediate return to the patient with no washing or cell concentration steps. Because of the lack of a washing step, this approach is used only for procedures that are expected to generate minimal debris.

In the operation of a blood salvage program, collaboration among surgeons, anesthesiologists, and transfusion medicine professionals is important because of the technical considerations as well as the selection of patients and equipment [76, 78, 79]. For instance, when blood is salvaged from noncardiovascular surgery in which the patients are not anticoagulated, the washing is particularly important, since the shed blood will usually contain activated coagulation factor proteins [80, 81]. The transfusion of these activated coagulation factor proteins can cause severe reactions. It is also essential to avoid hemolysis that can occur from improper use of the device such as high suction pressure and to avoid air embolus. Since the recovered shed blood will be primarily red cells when transfused, patients who receive large amounts of salvaged blood may develop a "depletion" coagulopathy even though they will have received little allogeneic blood. Blood replacement strategies should account for this possibility. Intraoperative salvage is contraindicated in patients with bacteremia or for salvaging blood from a surgical field thought to be contaminated. Tumor cells have been found in the cell salvage devices [82]. Intraoperative salvage is not used for surgeries involving malignancies because of the theoretical possibility of dissemination of tumor cells, although it has been suggested that intraoperative blood salvage could be done safely in many cancer patients [83, 84].

The potential complications of intraoperative blood salvage can be summarized as [76] coagulopathy, air embolus, infection, fat embolus, drug effects due to aspiration of drugs into salvage devices, and microaggregate effects. Because of the complexities of blood collection, storage, and transfusion and the patient selection necessary to operate a

safe and effective program, thorough procedures and documentation are important. Often blood bank personnel can work in collaboration with surgery personnel to develop and monitor the program and carry out ongoing quality control.

Intraoperative blood salvage has been used in cardiovascular, vascular, orthopedic, gynecologic, urologic [85], transplant (especially liver], and occasionally trauma patients [76]. In one study of cardiovascular surgery patients, blood salvage resulted in an overall reduction of 62% of the mean number of red cells transfused [86]. There are fewer studies of intraoperative blood salvage than for ANH, and those that are available are small and not well controlled. However, blood salvage is used more extensively than ANH, but the optimum conditions, the expected blood loss, and the devices used for each situation are not well defined.

6.6 Postoperative blood salvage

Following surgery, it may be possible to collect shed blood and return it to the patient in some circumstances. The most extensive experience with this is in cardiovascular surgery, although in the past few years interest has developed in salvaging blood from joints following joint replacement surgery. For total knee and total hip arthroplasty, collection of postoperative wound drainage with subsequent transfusion was shown to reduce the requirement for allogeneic red cells from 35% in a group of historical controls to 22% in 135 study patients [87].

Several devices are available for collection of blood, and these usually involve a chest tube drainage system with integral blood bag and filter. Blood salvaged from postoperative drainage is usually not anticoagulated. The obvious advantage is to obtain the patient's own shed blood for autologous transfusion. However, there are several concerns with postoperative salvage: (a) the wound drainage is usually dilute, and so the volume of red cells actually obtained may not be large; (b) the red cells in the drainage are usually partially hemolyzed; (c) there is activation of the coagulation system and the drainage contains activated coagulation proteins; (d) the drainage may contain cytokines; and (e) drainage may occur over a prolonged time, resulting in red cells that are damaged by exposure to room temperature. Because the postoperative salvaged blood is not washed before transfusion, the quality of the red cells being transfused may be poor or even dangerous for the patient. Thus, as for ANH, it is valuable for these procedures to be developed in collaboration with the blood bank and for personnel to be knowledgeable and to follow procedures so as to ensure the transfusion of a safe and effective red cell component.

6.7 Directed-donor blood

Directed donors are friends or relatives who wish to give blood for a specific patient. It is estimated that in 2006, a total of 70,000 units of blood

were donated as directed-donor units [6]. This represented 0.4% of the total available blood supply, which was a huge decrease from 1989. The appeal of directed-donor blood is that the patient hopes those donors will be safer than the regular blood supply. However, this blood might be less safe than the general community supply because the donors would be under considerable pressure to donate and might not be candid about their medical and risk-behavior history. In general, the data do not indicate that directed donors are either more or less safe than regular donors. Directed donors do not have a lower incidence of transmissible disease markers [88–90], and directed-donor blood is no safer than allogeneic blood donated for the general blood supply. Conversely, there is no evidence that the incidence of positive transmissible disease tests is increased in directed donors, which would cause directed-donor blood to be less safe. Thus, the transmissible disease testing data do not provide a factual rationale for directed donations. Despite this, directed-donor blood has considerable appeal to many patients, and it continues to be part of the blood supply for some hospitals. A few blood banks refuse directed donations, but most accept these donors as a service to the patients. Each hospital must also decide whether to sequester the blood and use it only for the intended patient or to allow the directed-donor blood to become part of the community's general blood supply if it is not used for the originally intended patient. If directed-donor units are to be "crossed over" into the general supply, the donors must meet all the usual FDA requirements for routine blood donation. In either situation, directed-donor blood requires additional attention and record systems for the blood center and hospital, thus increasing the cost and creating the possibility for errors to occur and the blood to be unavailable when desired for the particular patient.

6.8 Patient-specific donation

There are a few situations in which appropriate transfusion therapy involves collecting blood from a particular donor for a particular patient [91]. Examples are donor-specific transfusions prior to kidney transplantation, maternal platelets for a fetus projected to have neonatal thrombocytopenia, or family members of a patient with a rare blood type. In these situations, the donors must meet all the usual FDA requirements, except that they may donate as often as every 3 days so long as their hemoglobin remains above the normal donor minimum of 12.5 g/dL [26]. The units donated must undergo all routine laboratory testing.

6.9 Minimal donor exposure programs

Programs that attempt to limit the number of donors to which a patient is exposed usually involve pediatric patients because their size makes their blood requirements smaller than for most adults. Limiting the donor

exposures can be accomplished by some laboratory techniques, by selected-donor programs, and by the use of single-donor components.

Sterile connector devices can be used to allow multiple entries into a unit of red cells so that a patient can receive multiple transfusions of cells from the same donor [91–93]. The advantage of reducing donor exposures must be balanced against the use of blood that ages while being stored. Many pediatricians and neonatologists prefer to use blood less than 7–10 days old, especially for seriously ill neonates. This may limit the number of transfusions that can be provided from one unit of red cells even though the technology is available to enter the container under sterile conditions multiple times.

A mathematical model can be used to predict whether one unit of blood can be designated for a specific patient or one unit used to supply several patients [91]. This approach improves the efficiency of blood use but does not necessarily reduce the number of donor exposures. The Mayo Clinic physicians reported a unique program for children undergoing cardiovascular surgery, in which the parents donated platelets by apheresis before surgery; during surgery, blood salvage was used along with blood from one dedicated donor [92]. This reduced donor exposures by 80%. Strauss et al. [93] were able to obtain all of the blood needed by pediatric patients undergoing elective surgery from an individual donor dedicated to each patient by lowering the hematocrit necessary for donation. Thus, programs like these can result in reduction of donor exposures for some pediatric patients. The cost is probably higher than those of allogeneic donor programs, and the usual FDA donor requirements cannot be applied to donor selection. Very few such programs are in operation.

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7 Production of Components by Apheresis

Blood component therapy developed because of the use of plastic bag systems to allow separation of whole blood into some of its parts (see Chapters 1, 5, and 11). This led to important advances in hemotherapy and made possible many of the medical and surgical therapies used today. Techniques are also available to remove only the desired component and return the remainder to the donor, thus making it possible to process large volumes of donor blood and obtain a larger dose of the desired component from one donor. As early as 1914, Abel [1] removed whole blood, retained the plasma, and returned the red cells to the donor. During the 1950s and 1960s, apheresis procedures were developed using combinations of the plastic bags and tubing sets used for whole blood collection. A standard unit of blood was removed, the desired component (either plasma or platelets) separated, and the remainder of the blood returned to the donor; the process was repeated several times, thus producing a larger amount of the desired component than would have been obtained from one unit of whole blood [2]. The method was time consuming, cumbersome, and expensive; therefore, more automated methods were sought. The general establishment of the clinical effectiveness of transfusions in which all of the platelets were obtained from one donor (see Chapter 11) added momentum to the development of plateletpheresis procedures.

Semiautomated apheresis methods were developed generally by two separate research groups [3]. In Boston, the centrifuge apparatus developed for plasma fractionation by Edwin Cohen was modified to process whole blood from normal donors [4], and at the National Institutes of Health a blood cell separator was developed to aid in the treatment of leukemia [5]. Both of these approaches ultimately led to the sophisticated blood cell separators available today for the processing of large volumes of donor blood and the selective removal of the desired blood component. Thus, a major advance in the production of blood components and component therapy was the development and large-scale implementation of apheresis.

Apheresis, meaning to take away, refers to the process of selectively removing one component of whole blood and returning the remainder to the donor. The term plasmapheresis was used by Abel in 1914 to describe his initial work [1]. Today most apheresis is done using semiautomated instruments, sometimes called blood cell separators. In apheresis, the donor's whole blood is anticoagulated as it is passed through the

Table 7.1 Instruments available in the United States for collection of blood components by apheresis.

Instrument	Manufacturer	Platelets	Granulocytes	MNCs	PBSCs	Plasma	Red cells	Collected with plasma	Collected with RBCs
		Individual components produced						Combinations of components possible	
Spectra	Caridian	x	x	x	x	x		Platelets, MNCs	
Trima Accel	Caridian	x				x	x	RBCs, platelets	Platelets, plasma
Alyx	Fenwal						x	RBCs	Plasma
Amicus	Fenwal	x		x	x	x	x		Platelets
Autopheresis C	Fenwal					x			
Com.Tec	Fresenius	x	x	x	x	x		Platelets	
Cymbal	Haemonetics						x		
PCS2	Haemonetics					x			
MCS 8151	Haemonetics					x	x	RBCs	Plasma
MCS+ LN 9000	Haemonetics	x	x			x	x	Platelets	

Sources: From information provided by the manufacturers, and Burgstaler E. Blood component collection by apheresis. J Clin Apheresis 2006; 21:142–151.
MNCs, mononuclear cells; PBSCs, peripheral blood stem cells.

instrument where it is centrifuged and the blood is separated into red cells, plasma, and a leukocyte–platelet fraction. Then the desired fraction or component is removed, and the remainder of the blood is recombined and returned to the donor. Several liters of donor blood can be processed through the instrument, and therefore a larger amount of the desired component can be obtained than from one unit (450 mL) of whole blood.

There are several different instruments available for the collection of platelets, granulocytes, lymphocytes, red cells, peripheral blood stem cells (PBSCs), or plasma by apheresis (Table 7.1). All of the instruments used for normal-donor apheresis of cellular products use centrifugation to separate the blood components. Some operate in a continuous flow and others with intermittent flow; some require two venipunctures (an outflow and return) and others only one venipuncture. The instrument is operated by a microprocessor that controls the blood flow rate, the anticoagulant added to the whole blood entering the system, the centrifuge conditions, the component separation, and the recombination of the remaining components and returning them to the donor. For many years, blood cell separators were designed to collect one component (usually platelets) at a time. Recently, the approach has changed so that instruments can collect several different components either one at a time or in various combinations (Table 7.1). This is creating marvelous opportunities for more creative and efficient use of blood donations.

Pertinent comments about collection of each component are given below. For details of the operation of the instrument and collection

procedure, the manufacturer's instructions and references should be consulted.

Development of centrifugation instruments for cytapheresis

Intermittent-flow centrifugation

With the development of plasma fractionation during the 1940s, it was necessary to carry out continuous-flow washing of blood inside a bacteriologically closed centrifuge with no limitation of the volumes that could be processed. The special centrifuge system developed by Edwin Cohn (a professor of biochemistry at Harvard who originated the plasma fractionation procedure) was modified for use as a blood processor in collaboration with the Arthur D. Little Corporation (ADL) and one of its engineers, Allan Latham, and later James Tullis, MD, a Harvard hematologist [6]. The original motivation to modify the Cohn ADL bowl was for washing and deglycerolizing previously frozen red cells. However, because of the difficulty in obtaining an adequate supply of platelets, it soon became apparent that the Latham bowl could be used to separate whole blood and collect platelets [7, 8]. Soon a free-standing device, the Model 10, containing the centrifuge bowl was produced by Abbott Laboratories, but they did not choose to go into the business of manufacturing medical devices. The procedure was cumbersome because the bowl was made of stainless steel and had many parts, all of which had to be cleaned and sterilized between uses, and although it was a major innovation, it was not practical for routine or large-scale use. A new company called Haemonetics was formed and soon produced a more sophisticated instrument, the Model 30. The centrifuge bowl system was later made from Lucite and adapted to a special centrifuge [9] that became the Haemonetics system. This system was sterile, more self-contained, and included anticoagulant solutions, storage bags, and ancillary materials. Experience with this disposable plateletpheresis system was gained rapidly in many centers, and it became clear that a large number of platelets could be collected safely from volunteer donors [10–12].

Continuous-flow centrifugation

In the early 1960s, investigators at the National Cancer Institute (NCI) entered into collaboration with IBM Corporation to develop a device that could separate the cellular elements of blood on a continuous flow in vivo and return the plasma and red cells to the donor [13, 14]. This relationship developed because of the personal involvement of Mr. George Judson, an IBM engineer whose child was being treated at the NCI. The child was being treated by leukodepletion rather than chemotherapy, and collaborative efforts were made to develop an instrument for more efficient leukocyte removal. Supposedly, the first blood cell separator was constructed primarily of material obtained at a Bethesda, Maryland, hardware store [15]. Because the instrument was developed at the NCI, the

plans were in the public domain. The American Instrument Company obtained these plans and developed their version of the device, which they called the Aminco Celltrifuge.

During leukapheresis with these instruments, the donor would undergo venipuncture in each arm. Blood was pumped out of one vein and through the blood cell separator, where the granulocytes were removed, and the remaining blood was returned to the other arm. Blood flowed to the bottom of the centrifuge bowl by a central channel, flowed outward along the bottom, and up the sides where the red cells were packed against the walls. Blood separation occurred in a polycarbonate bowl with a clear plastic cover through which the operator could view the separation of plasma, buffy coat, and platelets. Each of these components was drawn off by a separate peristaltic flow pump adjusted by the operator to maintain optimum cell separation. Although the instrument was designed for granulocyte collection, it was also suitable for platelet collection [16]. The key to these instruments was the rotating seal, one section of which was attached to the rotating centrifuge bowl and the other fixed to the blood inflow and outflow lines [17]. The NCI-IBM Blood Cell Separator contained a blood reservoir so that the donor could be bled intermittently but blood flow into the centrifuge was continuous. The Aminco Celltrifuge was a more simple instrument without the reservoir system, but this necessitated continuous bleeding of the donor. These systems, like the original Latham bowl for plateletpheresis, were very cumbersome because they were made of multiple reusable parts that had to be cleaned, sterilized, and reassembled between procedures. To simplify the procedure and to use more disposable equipment, IBM developed the Model 2997 blood cell separator. In this instrument, the centrifuge bowl was replaced with a disposable hollow plastic blood separation channel attached at both ends to the input and output blood flow ports to form a closed loop [18]. This instrument then formed the basis for the development of the IBM, and later COBE (now Caridian), plateletpheresis instruments.

As the Latham bowl and the Haemonetics system were being developed for plateletpheresis, attempts were made to use this also for granulocyte collection. The intermittent-flow centrifuge was operated in much the same way as for plateletpheresis, but the operator then adjusted the blood flow rates and time of component collection to remove the buffy coat rather than the platelet layer [19–21].

Subsequently a new generation of apheresis instrument technology was developed [22, 23], including a microprocessor to control the operation of the instrument combined with a system that lacked the rotating seal present on the IBM, Celltrifuge, and Haemonetics devices. This system, the Baxter CS-3000, made possible different types of blood separation because different unique separation chambers were developed for the particular component desired [22, 23]. This system had the additional advantage of being completely closed and enabling sterile collection and thus storage of products for longer than the 24-hour limitation.

These three basic instruments—the Haemonetics models, the Gambro (COBE/Caridian) blood cell separator models, and the Baxter

SEPARATION CHAMBER

Platelet-rich plasma
Outlet port

Low "G" wall

Whole blood port

High "G" wall

PRBC Port

○ Platelets
● Whole blood cells
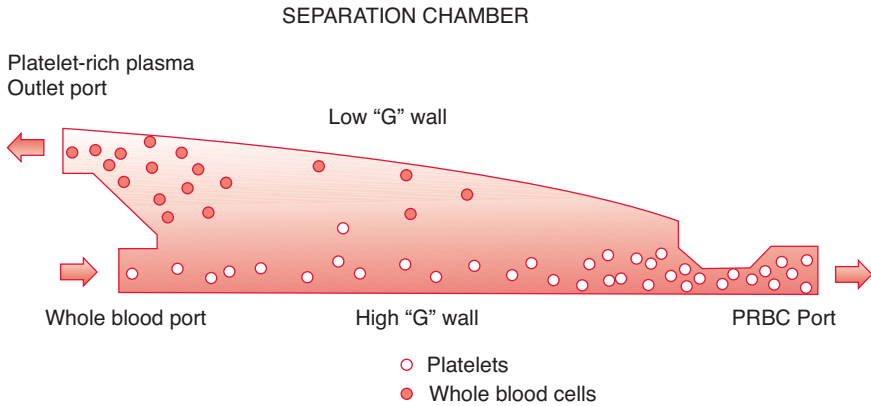
Figure 7.1 Flow pathway and blood separation of the Baxter Amicus Separator. (Courtesy of Fenwal, Inc.)

CS-3000—were the mainstay of apheresis for blood component production in the United States. Each instrument has certain strengths that make it advantageous based on the needs of the particular blood center [24, 25]. Recently, newer apheresis instruments have been developed that allow more convenient collection of different combinations of red cells, plasma, or platelets. The principle of each instrument will be described briefly.

The Fenwal CS-3000 and Caridian Spectra™ are being phased out and will not be discussed here.

Fenwal Amicus

The Amicus operates using a collection chamber and a separate component separation chamber (Figure 7.1). The centrifuge chamber design contributes to the fluid dynamics and component separation efficiency. Platelet-rich plasma from the collection chamber is continuously recirculated along with the whole blood entering the separation chamber to provide optimum blood component production. The Amicus can be used to collect platelets [26–28], PBSCs [29], or a combination of red cells, platelets, and plasma [30]. In plateletpheresis, the Amicus produces about 3.5×10^{11} platelets in 43 minutes [31]. For collection of PBSCs from patients–donors stimulated by chemotherapy and G-CSF, approximately 1.3×10^{10} mononuclear cells (MNCs) and 1.4×10^8 CD34+ cells can be obtained from an 8-liter blood processing procedure [32]. When concurrent red cells, platelets, and plasma are collected, the procedure produces 198 mL of red cells, 3.9×10^{11} platelets, and 198 mL of plasma in 74 minutes [30]. The red cells can be stored the usual 42 days when they are resuspended in an additive solution.

Fenwal Alyx

This multiple component collection system is continuous separation with fluid flows controlled by a pneumatic pump system using internal sensors

to monitor the weight of blood, fluids, and collection components [31, 33, 34]. The plastic disposable rigid-wall separation chamber and cassette interfaces with the pneumatic pump to control fluid flows. A leukodepletion filter is part of the system and the instrument automatically adds the red cell preservative. Although separation is continuous, blood flow from the donor is intermittent, with plasma being returned after withdrawal of about 300 mL of whole blood. The Alyx can produce two units of red cells in about 35 minutes [31]. The Alyx can also produce combinations of components.

Caridian Trima Accel[TM]

The Trima Accel, which operates on the basis of centrifugation, can be used for collection of platelets, plasma, or red cells in various combinations [27, 35–39] with a single-needle technique. The Trima Accel in about 56 minutes, compared with 80 minutes for the Spectra, to produce 3.5×10^{11} platelets [27]. Platelets pass through a leukoreduction filter before entering the storage bag. Red cells, platelets, and plasma collected using the Trima Accel have satisfactory in vitro characteristics, in vivo survivals, and in vivo clinical effectiveness [35–40].

Haemonetics instruments

The Haemonetics system uses a disposable, transparent Lucite centrifuge bowl for blood separation [9]. After venipuncture is performed and the donor is connected to the instrument, the operator activates the instrument and blood is pumped from the donor into the centrifuge bowl (Figure 7.2). Anticoagulant–citrate–dextrose (ACD) anticoagulant is added to the blood as it leaves the donor. The centrifuge bowl spins at approximately 4800 rpm and continuously separates the blood as it enters the bowl. When the volume of blood removed from the donor exceeds the capacity of the bowl, plasma begins to exit the bowl and is collected in a bag. The platelet/buffy coat layer accumulates at the top of the red cells, and as the bowl continues to fill, this layer moves toward the exit port. When the platelets—visible as a white band between the red cells and plasma—reach the exit port, a valve is activated, diverting the flow pathway into a separate bag, where the platelets are collected. When the platelets have been collected, the blood flow is stopped, the pumps reversed, and the plasma and red cells recombined and returned to the donor. This cycle of filling the centrifuge bowl is repeated several times to obtain the desired platelet yield.

The Haemonetics Multiple Component System (MCS and MCS+) have the flexibility to collect various combinations of platelets, plasma, and red cells [41, 42] (Table 7.1). The MCS and MCS+ can collect approximately 4 $\times 10^{11}$ platelets in 90 minutes [41, 42]. A combination of platelets and plasma can also be collected [43]. This exciting approach was finally the first step to provide flexibility to the donor center to determine on a daily basis or for individual donors the particular mix of components to collect.
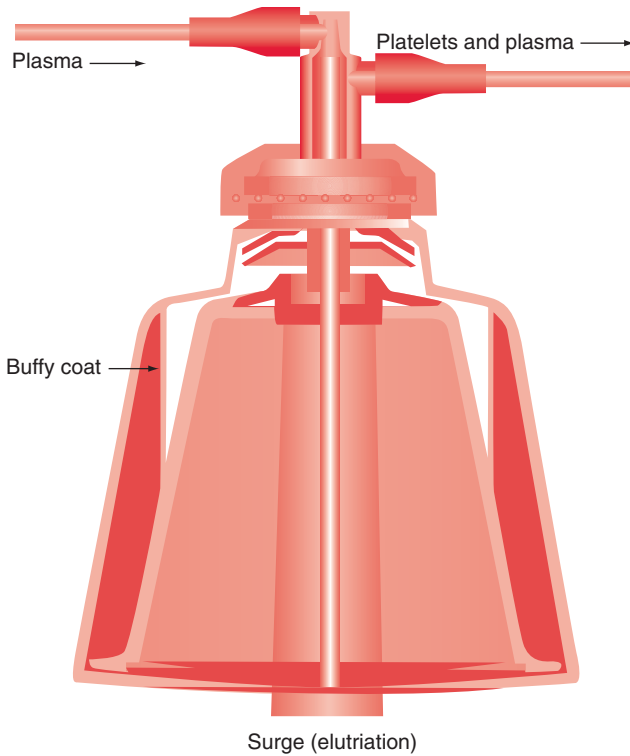
Figure 7.2 Flow pathway and blood separation in the Haemonetics Latham bowl system. (Courtesy of Haemonetics Corporation.)

Fresenius AS104

This instrument uses a separation chamber shaped like continuous spirals in which the flow path is interrupted by an integral barrier. There is a camera system that monitors the interface at every revolution of the separation chamber, and the plasma flow rate is automatically adjusted to maintain a constant hematocrit level in the separation chamber. Platelets are removed from the centrifuge as they are collected, and a target platelet yield can be set in the electronics of the system. This system conveniently produces a single-dose apheresis concentrate containing approximately 3.1×10^{11} platelets or double dose concentrate containing 5.29×10^{11} platelets [43]. The Fresenius instrument can also be used for blood stem cell collection [44].

7.2 Plateletpheresis for the production of single-donor platelet concentrates

The official Food and Drug Administration (FDA) name of this component is platelets, pheresis. In daily practice, this component is

usually called single-donor platelets or plateletpheresis concentrates. They are a suspension of platelets in plasma prepared by cytapheresis. A unit or bag of plateletpheresis concentrate must contain at least 3×10^{11} platelets in at least 90% of the units tested [45].

The use of apheresis, particularly for platelet production, is increasing substantially. In 1982, about 80,000 plateletpheresis procedures were performed [46] in the United States, and by 2006 this had increased to 1,167,000 yielding 1,823,000 apheresis products [47]. This accounted for 82% of all platelets produced [47]. The 2,396,000 platelets from units of whole blood and represents a 43% decrease of whole-blood platelets since 2004 [47].

Plateletpheresis usually requires about 1 1/2 hours and involves processing 4000–5000 mL of the donor's blood through the instrument. Platelets obtained by plateletpheresis are processed, tested, and labeled similar to whole blood (see Chapter 8). This includes ABO and Rh typing and testing for all required transfusion-transmitted diseases. The plateletpheresis concentrate may be stored for 5 days at 20–24°C if it is collected in a closed system. The number of platelets contained in each concentrate is determined, although this information may not necessarily be recorded on the label. Each platelet concentrate has a volume of approximately 200 mL and contains very few red cells (<0.5 mL), and so red cell crossmatching is not necessary. Quality control tests must show that at least 90% of the apheresis platelet concentrates produced by each facility contain 3×10^{11} platelets or more. The white blood cell content varies depending on the instrument and technique used for collection, but presently all plateletpheresis procedures produce leukodepleted platelets (<1×10^6 WBC).

The effects of plateletpheresis on donors are discussed in Chapter 4.

Function and storage of platelets obtained by apheresis

Platelets collected using these plateletpheresis systems have in vitro function and in vivo survival characteristics equal to platelets prepared from whole blood [10, 23, 36, 39, 48–52]. The platelets survived normally when autologous transfusions of radiolabeled platelets were given to normal research donors, and platelets collected by apheresis caused the expected increase in platelet count in thrombocytopenic patients [36, 39, 53]. The preservation medium and the size and composition of the storage container make it possible to store platelet concentrates produced by apheresis in a volume of about 200 mL for up to 5 days [54].

7.3 Collection of red cells by apheresis

Chronic shortages of group O red cells stimulated interest in the use of apheresis to collect the equivalent of two units of red cells from some donors, especially group O. Several instruments are now available for red cell apheresis [30, 31, 36, 38, 39, 55–60] (Table 7.1). The collection procedure is similar to other apheresis procedures except that red cells are

Table 7.2 Comparison of red cell units prepared from whole blood with red cell units prepared by double unit red cell apheresis.

	Whole blood	Alyx[a]	Trima	MCS[b]
Product volume (mL)	310	301	347	312
RBC volume (mL)	190	177	NA	182
Total hemoglobin (g)	55	57.8	60.7	—
Hematocrit (%)	60	58	55	58
Collection time (min)	8	28	NA	50

NA, not available.

[a]Louie J et al. Quality and characteristics of red cells collected on a new automated portable component collection system. Transfusion 2003; 43(Suppl):135A.
[b]Smith JW, Gilcher RO. Red blood cells, plasma, and other new apheresis-derived blood products: improving product quality and donor utilization. Transfus Med Rev 1999; 13:118–123.

retained rather than being returned to the donor. Additional saline may be infused to the donor to maintain blood volume. The red cells usually have a very high hematocrit as they are removed from the instrument, but the red cells can be stored in an additive solution for the usual 42 days [30, 36, 39, 59]. The red cell products obtained by apheresis are much more standardized than red cells prepared from whole blood, but otherwise red cells obtained by apheresis have the same characteristics as those produced from whole blood (Table 7.2). The advantages provided by red cell apheresis are to obtain two units of red cells from one donation to allow for fewer donor visits, possible increases in red cell availability, and potentially fewer donor exposures if both units of red cells from one donor are transfused to one patient. Donors for two-unit red cell apheresis must meet weight and hemoglobin standards specified for each instrument. Since two units of red cells are removed, they may donate only every 4 months. This is adequate for red cell recovery but may not allow complete regeneration of iron stores [61]. Apheresis for two-unit red cell collection is taking its place in the mixture of blood component production activities (Table 7.2). Although reactions following RBC collection are more common than whole blood donation, almost all reactions were minor and for donors less than 20 years, reactions are equally common after two RBC or whole blood. Thus, two RBC collections are as safe as whole blood [62].

7.4 Leukapheresis for the production of granulocyte concentrates

Leukapheresis produced only a marginally adequate dose of granulocytes for therapeutic benefit and never gained wide use (see Chapter 11). There is a resurgence in leukapheresis now that G-CSF can be used to elevate the donor's granulocyte count and increase the yield of granulocytes (Chapters 11 and 17).

This blood component is called granulocytes, pheresis. The component is a suspension of granulocytes in plasma prepared by cytapheresis. A granulocyte concentrate must contain at least 1×10^{10} granulocytes in at least 75% of the units tested [45]. Neither the American Association of Blood Banks Standards nor FDA regulations specify the number of units that must be tested for quality control purposes. However, since only a few granulocyte concentrates are prepared by most blood banks, it is customary to test all concentrates.

Leukapheresis procedures are usually more complex and lengthy than plateletpheresis. The leukapheresis procedure takes 2–3 hours, compared with about 1 1/2 hours for plateletpheresis, to process more blood and improve the granulocyte yield. Usually 6500–8000 mL of the donor's blood is processed through the instrument, with removal of about 50% of the granulocyte, resulting in a granulocyte concentrate with a volume of about 200 mL. Because granulocytes do not completely separate from the red cells, granulocyte concentrates usually contain a substantial amount of red cells (hematocrit 10% or about 20 mL of red cells), and therefore red cell crossmatching is necessary. The granulocyte content of each concentrate is determined, but not necessarily indicated, on the label.

Because of the relatively low level of circulating granulocytes in normal donors, it was necessary to process a large volume of blood to obtain a usable dose of granulocytes. Initial development of the instruments and experience with granulocyte transfusions involved collecting cells from patients with chronic myelogenous leukemia (CML). However, there were the obvious problems of the use of abnormal or malignant cells and the limited number of CML patients available to donate. The two additional strategies used to increase the granulocyte yield are the addition of the blood sedimenting agent hydroxyethyl starch (HES) to improve granulocyte separation within the centrifuge and the treatment of donors with corticosteroids, and more recently with G-CSF, to increase the level of circulating granulocytes (see Chapter 17).

Hydroxyethyl starch in leukapheresis

The separation between granulocytes from the upper layer of red cells is poor because the density of granulocytes is similar to that of some red cells. Although several agents can be used to sediment red cells in vitro, HES is used because it is licensed in the United States for in vivo use and is not associated with unacceptable reactions or alteration of coagulation tests. The granulocyte yield is doubled when HES is added to the leukapheresis system by constant infusion [63–65]. Several studies of the effects of HES established that the nature and incidence of reactions are acceptable for use on normal donors, the potential for blood volume overload when administered to normal donors can be easily managed during the procedure, there is no adverse affect on laboratory values or platelet or granulocyte function, and there are no adverse long-term effects. Pentastarch has a shorter in vivo half-life than HES and can also be used in leukapheresis [66, 67].

Stimulation of donors with corticosteroid or G-CSF prior to leukapheresis

The second approach to increase the granulocyte yield is to increase the donor's circulating granulocyte count. Corticosteroids seemed to be the drug of choice, and dexamethasone was selected because it could be given either orally several hours before leukapheresis or parenterally at the beginning of the procedure. Dexamethasone 60 mg can be given orally the evening before, or hydrocortisone 4 mg/m^2 can be given intravenously 6–12 hours before leukapheresis. This is a very effective method to increase the granulocyte yield even further than is accomplished by adding HES to the separation system [64]. It has been suggested that corticosteroids may cause cataracts in granulocyte donors [68], although this was not substantiated in a larger study [69]. Granulocyte colony stimulating factor (G-CSF) has also been given to normal donors to increase the peripheral granulocyte count to improve the yield of granulocytes for transfusion. Depending on the dose schedule, the granulocyte count increases to between 20,000 and 40,000 per microliter after several days of G-CSF treatment [70–74]. Using G-CSF-stimulated normal donors, it is possible to obtain granulocyte concentrates containing about 4×10^{10} granulocytes or more [72–75]. More recently, use of dexamethasone has been combined with G-CSF to provide even higher granulocyte levels in the donor, resulting in granulocyte concentrates containing up to 6×10^{10} granulocytes [72]. A large multi-center randomized trial is now underway to evaluate these high-dose granulocyte concentrates.

Filtration leukapheresis (FL)

This method of granulocyte collection is described because of historical interest, but it is not used today. A nylon fiber filter system was developed to collect granulocytes [76]. Although this system yielded a larger number of cells than the centrifuge procedures, granulocytes obtained by FL had a mild to moderate functional impairment and decreased intravascular recovery and survival [77, 78]. Also, a severe transient neutropenia occurred a few minutes after the donor's blood came in contact with the nylon fibers [78–81] due to activation of the complement system [81, 82]. Reports of donor complications [83] led to the discontinuation of FL.

Function of granulocytes obtained by leukapheresis

Granulocytes collected by centrifuge leukapheresis techniques demonstrate normal bacterial killing, phagocytosis, granulocyte metabolism), chemiluminescence, superoxide production, and chemotaxis [77, 84–86]. In vivo studies using isotope-labeled cells showed that granulocytes a normal intravascular recovery and survival and migrated to sites of inflammation [77, 87–89]. The use of corticosteroids or G-CSF in donors to improve the granulocyte yield does not adversely affect the function of the cells in vitro or in vivo [75, 82, 84, 87].

Storage of granulocytes for transfusion

Granulocytes have a life span in the circulation of only a few hours, and so storage of granulocytes as part of a routine blood bank operation is difficult. Granulocytes retain bactericidal capacity and metabolic activity related to phagocytosis and bacterial killing for 1–3 days of storage at refrigerator temperatures, although chemotactic response declines by 30–50% after 24 hours [88–91]. Studies using ^{111}In-labeled granulocytes showed that storage of granulocytes between 1°C and 6°C for 24 hours was associated with a reduction in the percentage of transfused cells that circulated and about a 75% reduction in migration into a skin window [88], but storage at room temperature for 8 hours did not reduce the intravascular recovery, survival, or migration into a skin chamber [88]. In vivo recovery, survival, or migration was reduced further when granulocytes were stored longer than 8 hours at room temperature or for even 8 hours between l°C and 6°C. Thus, it appears that granulocytes can be stored for up to 8 hours at room temperature before transfusion. Granulocyte concentrates from G-CSF contain large numbers of granulocytes with increases in IL-1B, IL-8, and decreases in pH during storage [75]. Thus, storage of granulocyte concentrates obtained from G-CSF stimulated donors is probably even less effective than the above data indicated. It is recommended that granulocytes be transfused within a very few hours. AABB standards allow storage for up to 24 hours at 20–24°C [45].

Donor–recipient matching for granulocyte transfusion

ABO antigens are probably not present on granulocytes (see Chapter 9), but granulocyte concentrates must be ABO-compatible with the recipient because of the substantial volume of red cells in the concentrates. The clinical impact of ABO incompatibility on granulocyte transfusion was evaluated in one study [92]. A small number of ^{111}In-labeled granulocytes free of red blood cells were injected into ABO-incompatible recipients. The intravascular recovery, survival, and tissue localization of the cells were not different from those seen when similar injections were given to ABO-compatible subjects [92]. This study was not intended to encourage the use of ABO-incompatible granulocyte transfusions, but this could be considered if granulocyte concentrates that are depleted of red blood cells could be prepared.

Incompatibility by leukoagglutination or lymphocytotoxicity was associated with the failure of transfused CML cells to circulate or localize at sites of inflammation [93–95]. Studies using ^{111}In-labeled granulocytes in humans established that granulocyte-agglutinating antibodies were associated with decreased intravascular recovery and survival, failure of the cells to localize at known sites of inflammation [95], and excess sequestration of transfused granulocytes in the pulmonary vasculature [95, 96]. However, applying these research data to the practical operation of a blood bank and granulocyte transfusion service is difficult. Granulocytes can be stored for only a few hours, and cells are not usually available for crossmatching to allow advance selection of compatible donors. The only

practical approach has been to screen the patients' serum against a panel of cells periodically to determine whether the patient is alloimmunized. If so, HLA-matched unrelated donors or family members can be selected for leukapheresis. However, the problem of donor–recipient matching and compatibility testing for granulocyte transfusion has never been solved.

7.5 Lymphocytapheresis for the collection of mononuclear cells

Lymphocytes or monocytes are being used increasingly as starting material for the production of cells for adoptive immunotherapy or as a concentrate enriched in PBSCs (see Chapter 18). Lymphocytapheresis also may be done as a therapeutic procedure to treat immune diseases by the physical removal of lymphocytes (see Chapter 19).

Because of the reasons lymphocytes are being collected, lymphocytapheresis is almost always done on patients. Thus there are no established criteria for normal donor selection and management. Lymphocytes can be collected using the Caridian Spectra, Baxter or Amicus, or Haemonetics instruments. Each system has a mononuclear cell collection procedure that very efficiently yields $1–3 \times 10^{10}$ MNCs.

7.6 Cytapheresis for the collection of peripheral blood stem cells

Hematopoietic stem cells are present not only in the marrow but also in the peripheral circulation and can be collected by cytapheresis. Normally the number of circulating PBSCs is small—much less than in the marrow. However, after the marrow suppression of chemotherapy, there is a rebound and the number of PBSCs increases substantially. This makes it possible to collect PBSCs from patients undergoing chemotherapy, especially for malignancies in which there was suspected marrow involvement, thus making the marrow unsuitable for autologous transplant. The PBSCs—expected to contain few, if any, malignant cells—can be used as marrow rescue following the chemotherapy. These autologous transplants of PBSCs made new chemotherapy regimens possible and also established that PBSCs could be used successfully for autologous marrow transplantation [97–102].

For several years, use of PBSCs was limited to autologous transplants. It was feared that the large number of T-lymphocytes contained in the PBSC concentrates would cause severe graft versus host disease and that T-depletion would result in an unacceptably large loss of PBSCs. However, this did not occur [102–107]. PBSCs result in more rapid engraftment [108], give results equivalent to marrow [107, 109] and may provide faster lymphocyte return, resulting in fewer infections [110]. Thus, considerable interest has developed in the methods to obtain PBSCs from both patients and normal donors.

PBSCs can be obtained from the peripheral blood by apheresis, but due to the small number of circulating PBSCs, multiple procedures would be necessary to obtain enough cells for transplantation. To further increase the level of circulating PBSCs, donors are given the growth factor G-CSF. In studies of normal subjects, the administration of G-CSF causes an increase in the percentage of CD34+ cells from 0.05% before treatment to about 1.5% after 5 days [111–113]. This results in a yield of about 4.5×10^8 CD34+ cells from a single apheresis [112]. The usual dose of CD34+ cells considered suitable for transplantation is about $2.5–5 \times 10^6$/kg or about 2×10^8 for a 70-kg person. Thus, one such apheresis concentrate is usually adequate for a transplant. Another approach to reducing the number of apheresis procedures necessary is large-volume leukapheresis, in which 15 or more liters of donor blood are processed to increase the number of PBSCs obtained [114]. Thus, PBSCs are a new hematopoietic progenitor component for allogeneic stem cell transplantation, and collection of PBSCs from normal donors now exceeds marrow in many transplant centers [73, 112, 115, 116], thus eliminating marrow collection in the operating suite, along with the attendant risks of anesthesia and the marrow collection process.

Collection procedures

PBSCs can be collected using the Caridian Spectra, the Baxter CS-3000, or the Baxter Amicus instruments (Table 7.1). The procedures are the same or similar to those used for mononuclear cell collection. The CS-3000 procedure uses the TNX upgrade and unique program settings and modified interface detection settings and centrifuge speeds [114, 117]. The granulocyte separation chamber and the small-volume collection chamber are used. In the Gambro Spectra, the mononuclear cell procedure is used for PBSC collection. The operator enters the donor's hematocrit, height, weight, and gender, and a microprocessor controls the addition of anticoagulant and the separation process. Because the cell separation depends on centrifugal force and dwell time in the gravitational field, the microprocessor varies the centrifuge speed if the blood flow rate varies. The operator can customize the procedure if desired, and the separation and actual collection of the PBSC component are determined by the operator observing the interface between red cells and buffy coat. During collection, the operator monitors the collection interface and the color of the collection material to optimize the resulting PBSC concentrate. PBSCs can be collected from small children [118], but adjustments are necessary in priming the system and managing the donors. PBSC collection with the Amicus involves cycles of filling the separation and collection chambers using computer software designed for PBSC collection. The operator determines the number of cycles, cycle volume, flow rate, RBC offset valve and interface set point. In a direct comparison of PBSC collection using the Spectra, CS-3000, and MCS 3P, Hitzler et al. [119] found no difference in the number of CD34+ cells in the products, although the Spectra more efficiently removed CD34+ cells from the donor and thus provided cells in the shortest time. Ikeda et al. [32] found no differences in cell collection

between the Amicus and Spectra, and yields were similar to historical yields from the CS-3000. Morton et al. [120] found the Caridian Spectra to be superior to the Haemonetics MCS-3P. Thus, it appears that all three of these instruments can be used for PBSC collection and the choice will be based on local factors.

For normal donors, the usual skin preparation, venous access, needles or catheters, solutions, and software are used. Blood flow rates of 40–80 mL/minute are used depending on the donor's venous access and blood flow tolerance. The mononuclear cell collection procedures involve processing 10–15 L of blood over 2 or 3 hours, although usually a larger volume of blood is processed in order to increase the PBSC yield [114]. There may be recruitment of CD34+ cells during extended apheresis up to 40 L over 5 hours. However, it is not clear that the CD34+ cell levels remain stable or increase (recruitment) during apheresis of normal donors, and so most centers process 15–18 L of blood, and this usually provides a suitable dose in one or two procedures.

Effects of PBSC collection on normal donors

The major clinical effects of PBSC collection on donors are caused by the G-CSF the donors receive to mobilize the PBSCs. Almost all donors experience some side effect [73]. The most common of these is bone pain, but headache, fatigue, and flu-like symptoms also occur. The donor's leukocyte count increases to 30,000–40,000 per microliter and the platelet count decreases by about 40% [120, 121]. The leukocyte and platelet counts return to normal by about day 16, or about 10 days after the apheresis donation and discontinuing the G-CSF. There is an increase in alkaline phosphatase, alanine aminotransferase, lactate dehydrogenase, and sodium and a decrease in glucose, potassium, bilirubin, and blood urea nitrogen. In donors who receive G-CSF the spleen size increases [122] and splenic rupture has been reported [123]. Although most donors experience some side effects, these are mild and should interfere with PBSC donation only rarely.

Characteristics of the PBSC concentrates

Large quantities of CD34+ cells can be collected from normal donors given G-CSF for 5 days. G-CSF doses of 7.5 or 10 mg per day provide a greater yield than 5 mg per day [115], but it is not clear whether there are statistically significant differences in the CD34+ cell yield between 7.5 and 10 mg per day. Because the donor side effects increase with increasing doses of G-CSF, we recommend that donors be given 7.5 mg of G-CSF per kilogram per day to mobilize peripheral blood CD34+ cells [115]. The composition of the PBSC component is shown in Tables 7.3 and 7.4. Most PBSC components contain only a small volume of red cells (mean, 7 mL) but a rather large total number of neutrophils and platelets [115]. This number of platelets is similar to the number of platelets ordinarily provided in a platelet transfusion, and this may provide an additional benefit from the PBSC transfusion. The PBSC concentrate usually has a

Table 7.3 Effects of 10 μg/kg G-CSF dose on the quantity of cells collected by one apheresis procedure from healthy people treated with G-CSF for 5 days (number of cells collected).

All components ($n = 150$)			
Cell type	Mean ± SD	Median	Range
WBCs ($\times 10^9$)	39.8 ± 21.8	36.0	15.6–163.3
MNCs ($\times 10^9$)	38.1 ± 19.4	34.5	15.6–139.7
CD34+ cells ($\times 10^8$)	452 ± 294	383	78–1380
CD34+ cells ($\times 10^6$ per L processed)	53.2 ± 33.1	46.5	9.3–146.3
RBC (mL)	7.2 ± 3.5	7.6	0–15.5
Neutrophils ($\times 10^9$)	1.77 ± 3.37	1.05	0–23.68
Platelets ($\times 10^{11}$)	490 ± 100	490	250–740

Source: Stroncek DF, Clay ME, Smith J, et al. Composition of peripheral blood progenitor cell components collected from healthy donors. Transfusion 1997; 37:411–417.

volume of about 200 mL and contains approximately 3×10^{10} MNCs and 4×10^8 CD34+ cells.

The total number of CD34+ cells collected is similar for the Caridian Spectra and the Fenwal CS-3000, but the composition of the components is slightly different (Table 7.5). PBSCs collected using the CS-3000 contained more MNCs, and those collected using the Spectra contained more granulocytes. The PBSC concentrates collected with both instruments contained a similar number of CD34+ cells and platelets. The instruments seem to perform similarly, and local operator or institutional preferences may make one or the other more desirable.

Quality control of PBSC concentrates

Since there is no definitive test for the primordial hematopoietic stem cell, quality control of these PBSC concentrates is not standardized. Cell culture techniques can be used to determine CFU-GM, BFU-E, and CFU-MIX colonies, and the number of CD34+ cells can be determined by flow

Table 7.4 Quantity of cells in the PBSC components.

Cell type	CS-3000 ($n = 15$)	Spectra ($n = 14$)	P
WBC ($\times 10^9$)	40.9 ± 21.7	33.1 ± 10.7	0.24
Neutrophils ($\times 10^9$)	1.38 ± 1.88	5.53 ± 8.71	0.001
Mononuclear cells ($\times 10^9$)	39.6 ± 21.9	26.9 ± 5.6	0.02
Platelets ($\times 10^9$)	507 ± 98	531 ± 116	0.54
CD34+ cells ($\times 10^6$)	470 ± 353	419 ± 351	0.69

Source: Stroncek DF, Clay ME, Smith J, Jaszcz WB, Herr G, McCullough J. Comparison of two blood cell separators in collecting peripheral blood stem cell components. Transfus Med 1997; 7:95–99.

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Platelets ($\times 10^9$)	507 ± 98	531 ± 116	0.54
CD34+ cells ($\times 10^6$)	470 ± 353	419 ± 351	0.69

Source: Stroncek DF, Clay ME, Smith J, Jaszcz WB, Herr G, McCullough J. Comparison of two blood cell separators in collecting peripheral blood stem cell components. Transfus Med 1997; 7:95–99.

cytometry. In practice, the dose for transplantation is usually based on cell counting to obtain at least 3×10^8 mononuclear or 5×10^6 CD34+ cells per kilogram of the recipient's body weight. The results of progenitor assays are not available for about 2 weeks and thus can be used only in retrospect for research purposes. Many centers do not even determine progenitor content as they do not believe there is a correlation with engraftment, although we believe this can be a valuable quality control test.

There is considerable variation in the number of CD34+ cells collected (Figure 7.3). In our early experience [115], a single-cytapheresis procedure yielded a median dose of $780–1658 \times 10^6$ CD34+ cells. In approximately 42% of the procedures, this would be an adequate cell dose to transplant 5×10^6 CD34+ cells per kilogram to a 75-kg recipient. In 86% of donors, two cytapheresis procedures would yield an adequate cell dose for transplanting the 75-kg recipient. These numbers were obtained by processing approximately 10 L of whole blood, and most centers now process 15–20 L. Other reports involving processing of 15–20 L of blood for each cytapheresis procedure suggest that larger numbers of CD34+ cells are obtained [114]. Thus, presently for most donors, one or two procedures result in a dose of cells suitable for transplantation.

Storage of PBSCs

Because of the variability in the number of cells that may be obtained, the strategy for using the cells for transplantation cannot always be the same. If the dose needed for transplantation can be obtained with one procedure, the cells can be transfused immediately. However, if two or three apheresis procedures are necessary, it may be desirable to freeze the concentrates and transfuse them all at once. However, the freezing and thawing may alter the composition of the PBSC concentrates, and so some transplant physicians give the cells fresh each day until the desired dose is obtained. Alternatively, the concentrate collected on the first day is stored in the liquid state and transfused with the concentrate collected on the second day. It appears that PBSCs can be preserved satisfactorily in Plasmolyte A, Normosol or STM-Sav for 24 hours at room temperature [124]. A more extensive discussion of hematopoietic stem cell preservation is in Chapter 18.
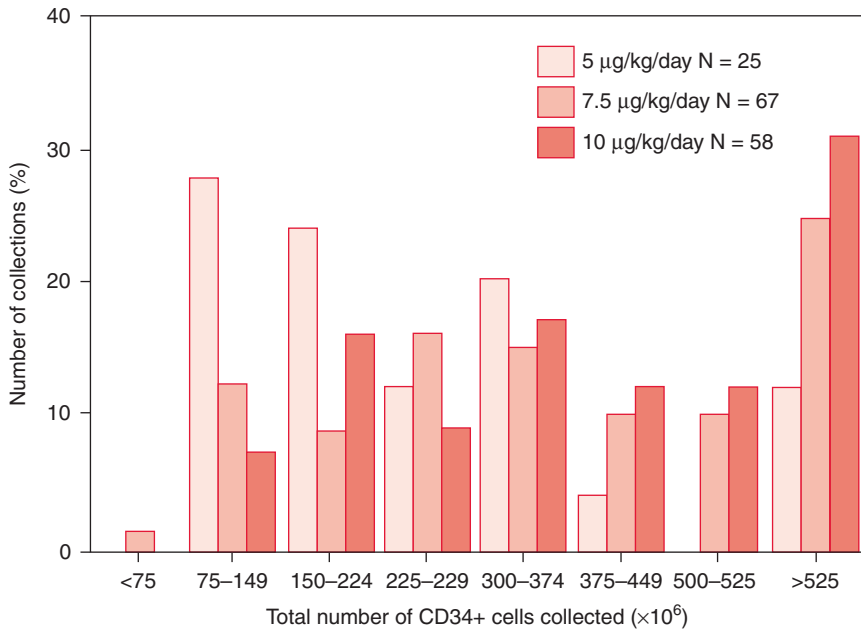
Figure 7.3 CD34+ cell yield in peripheral blood concentrates collected from normal donors. (Reproduced with permission from Stroncek DF, Clay ME, Smith J, et al. Composition of peripheral blood progenitor cell components collected from healthy donors. Transfusion 1997; 37:414.)

7.7 Donor selection and complications of cytapheresis in normal donors

Because donation of blood components by apheresis is fundamentally different than whole blood donation, there are some donor eligibility requirements and complications that are unique to apheresis donors. This chapter focuses on the donation procedures and the products. Donor selection and complications are discussed in Chapter 4.

7.8 Plasmapheresis and source plasma

The plasma collection and fractionation industry in the United States developed during the 1960s using manual plastic bag methods for plasma collection by plasmapheresis. Today, virtually all source plasma collected in the United States for fractionation into derivatives (Chapters 2 and 5) is obtained by semiautomated instrument plasmapheresis. It has been estimated [47, 125] that about 28 million liters of plasma are fractionated annually in the world (see Chapter 2). Most plasma used as fresh frozen plasma (FFP) is obtained from whole blood, but the increasing flexibility

of some apheresis instruments makes it possible to obtain plasma for FFP as a byproduct of platelet or red cell apheresis. There are no data on the number of plasma products produced in this manner. Apheresis plasma contains greater activities of factor V, factor VIII, factor IX, and factor XI, prothrombin fragments 1 and 2 and platelet factor IV compared with recovered plasma (see Chapter 4 and references [125, 126]). Thus, apheresis appears to produce plasma with a higher quality although the clinical significance of this is not established.

Source plasma is the starting material for the further manufacture of some diagnostics and plasma "derivatives." Derivatives are described in more detail in Chapter 2, and the selection and medical evaluation of plasma donors is described in Chapter 4. Plasmapheresis was done using sets of multiple plastic bags and involved separation of the blood from the donor such that there was a chance for return of red cells to the incorrect donor. Source plasma is now collected by semiautomated instruments that require less operator involvement, while producing larger amounts of plasma at a reasonable cost. The instruments in use in the United States are the Fenwal Autopheresis C and the Haemonetics PCS. The Haemonetics PCS can be used to collect platelet-poor or platelet-rich plasma. Usually one venipuncture is used and the system can be set up in about 5 minutes. This includes loading the disposable plastic set into the instrument, connecting the anticoagulant and solution bags, recording appropriate data, and placing the collection bags. The venipuncture area is prepared as for whole blood collection (see Chapter 4), and the venipuncture is done using the needle integral with the disposable plastic set used for the procedure. The operator then activates the instrument, and blood flow is initiated by the pumps in the instrument. Anticoagulant is metered into the blood flowing into the instrument in the proper ratio, and the centrifuge bowl is filled until the optical sensor detects the red cell interface and stops the inflow of blood. During this filling phase of the cycle, the plasma has been diverted into the collection bag. After the plasma–cell interface has reached the detector, the blood flow is reversed and the red cells are pumped from the bowl back to the donor. The cycle is then repeated until the desired amount of plasma is obtained. Usually about 500 mL of plasma can be obtained in about 30 minutes [127]. These instruments might be used to produce FFP but are not used extensively to produce source plasma.

The Baxter Autopheresis C plasmapheresis instrument operates on a different principle from the Haemonetics devices. The Autopheresis C combines filtration and centrifugation to separate blood in a smaller chamber (and possibly more efficiently). The instrument setup and donor preparation are the same as described for the Haemonetics systems and for whole blood collection. For the Autopheresis C, blood is withdrawn from the donor into a closed, disposable plastic set with a total extracorporeal volume of about 165 mL. Blood separation occurs in a small (7-mL) cylinder that is part of the system. A magnet causes rotation of the cylinder inside a larger compartment. The cylinder is composed of a membrane, and as the cylinder rotates, plasma moves peripherally through the membrane, thus providing the filtration part of the separation system.

The system does not operate in a continuous-flow manner; blood is returned intermittently to the donor through the single venipuncture and the process is repeated. This system also collects about 500 mL of plasma in about 30 minutes [128, 129]. The Autopheresis C is used extensively for the production of source plasma for further manufacture of plasma derivatives.

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8 Laboratory Testing of Donated Blood

Each unit of whole blood or each apheresis component undergoes a standard battery of tests (Table 8.1) to determine the red cell phenotype, the presence of red cell antibodies, and test for transmissible diseases. Additional tests such as those for cytomegalovirus, HLA antibodies, IgA levels, or rare red cell phenotypes may be performed as an option. The total number of test results for each unit of donated blood has increased substantially since the late 1980s. Since each unit of whole blood is separated into several components and there is a donor history record and two or three tubes of blood for tests, each donation generates many different data elements. Each of these must be properly identified and all data amalgamated to ensure that all testing and donor-related information is complete and the results are satisfactory before the blood or components can be released into the transfusion inventory. Since busy blood collection centers deal with hundreds of donors each day, sophisticated computer systems are used and, where possible, automated laboratory testing equipment is integrated into these systems.

ISBT 128 is a new global standard for the identification, labeling, and information processing of human blood, tissue, and organ products across international borders and disparate health care systems. It involves a unique, highly flexible and comprehensive coding method for every collected product and provides international consistency to support the transfer, transfusion, or transplantation of blood and tissue products. Thus, the modern blood center uses pharmaceutical-type manufacturing processes to ensure accuracy [1–3] (see Chapter 20) and cost-effectiveness.

8.1 Red cell blood group testing

Testing of donated blood is carried out on blood specimens obtained for this purpose at the time of donation. The blood is collected in separate tubes or a pouch in the tubing through which the blood passes. The maximum volume of blood that can be retained for testing is about 30 mL.

ABO typing
The most common cause of a fatal transfusion reaction is the administration of ABO-incompatible red cells (see Chapter 14). Therefore,

Table 8.1 Tests of donor blood.

Blood grouping tests
  ABO typing
  Rh typing
  Red cell antibody detection
Transmissible disease testing
  Treponemal antigen
  Hepatitis Bs antigen
  Hepatitis Bc antibody
  Hepatitis C antibody
  HCV antigen
  HIV 1 and 2 antibody
  HIV antigen
  HTLV-I and -II antibody
  WNV[a]
  Bacteria (platelets)[a]
Optional tests
  Cytomegalovirus[a]
  Platelet antigen typing[a]
  Rare red cell antigens[a]
  IgA levels[a]
  Trypanosomia cruzi[a]

HCV, hepatitis C virus; HTLV-I, human T-cell lymphotrophic virus type I; HTLV-II, human T-cell lymphotrophic virus type I; WVN, West Nile virus.
[a]Not required by FDA.

the ABO and Rh typing of the donor units is of critical importance. Usually ABO and Rh typing are carried out together, along with red cell antibody detection testing, since all of these tests involve red cell antibody–antigen interaction. The ABO and Rh typing can be done in a variety of systems, including slides, tubes, solid-phase microplates, gel systems, or affinity columns. Complex semiautomated instruments are available for typing large numbers of blood samples (Table 8.2). These instruments handle the specimen from start to finish, adding reagents, carrying out the incubation, reading the reaction, and providing the result, which can be interpreted by the computer in the instrument or provided for manual interpretation. The choice of the particular method and instrument will depend on the specific circumstances at the donor center testing laboratory. There are some similarities in methodology among all of the test system configurations. Antisera or reagent red cells are added, and tests of both red cells and serum are carried out at room temperature. The cell suspension is usually centrifuged or manipulated in some way to foster agglutination. An effort is made to disperse the cell suspension, and the resulting mixture is observed either visually or by an instrument.

For ABO typing, both the red cells and the serum are always tested. The red cells are tested with anti-A, anti-B, and anti-A, B (group O) sera. The A, B (group O) serum is used to detect weak subgroups of A (see Chapter 9), because the anti-A or anti-B in serum from type O individuals

Table 8.2 Automated systems for red cell typing and antibody testing of donated blood[a].

Company	Product	Type of Assay
Immucor	Gallileo	Solid-phase test
Immucor	Neo	Solid-phase test
Immucor	Echo	Solid-phase test
Bio-Rad	Tango	Solid-phase test
Ortho	Provue	Gel-phase test
Ortho	ID-MTS Gel Workstation	Gel-phase test
Ortho	AutoVue Innova	Column-agglutination test (glass beads)

[a]All of these systems can also be used for crossmatching.

reacts more strongly with some weak subgroups than anti-A or anti-B sera. Some of the weak subgroups of A require incubation before a reaction can be seen, and they may be missed in routine testing. Many blood typing reagents are monoclonal antibodies. These reagents have much greater specificity and strength of reactivity than older reagents that were human allosera. In addition to testing the red cells with antisera, the serum of each donor is tested against A and B red cells to determine which, if any, ABO antibodies are present. The group A test red cells should be A1 to provide the strongest reactions. Commercial reagent A and B cells are almost, if not always, Rh negative. This is to avoid apparent false-positive reactions in the ABO typing tests if anti-Rh antibodies are present in the donor and react with the A or B Rh-positive reagent red cells. The ABO typing is done using both red cells and serum to take advantage of the known relationship of ABO antigens and antibodies to strengthen the validity of the test result. Any discrepant test result must be resolved before the donor unit can be labeled and released from quarantine into the usable inventory. The common causes of ABO typing problems are listed in Table 8.3.

Rh typing

There are many antigen specificities in the Rh system, but the D antigen is highly immunogenic, and alloantibodies to the D antigen can cause severe or fatal hemolytic disease of the newborn or transfusion reactions (see Chapter 9). Because of the clinical importance of alloantibodies to the Rh(D) antigen, all donated blood is typed for this antigen but not others within the Rh system. This makes it possible to select Rh-negative red cell components for patients who are Rh negative. Most Rh-negative individuals do not have circulating anti-D, and so the combination of cell and serum typing done for ABO is not done for Rh. Rh typing reagents have been more complex than ABO reagents. Because ABO antibodies are usually a combination of IgG and IgM and cause direct agglutination at room temperature, it is possible to carry out ABO typing tests without extensive incubation or the need for agents to potentiate the antigen–antibody reaction. However, anti-D is usually an IgG-type antibody that does not cause direct agglutination at room temperature.

Table 8.3 Common causes of ABO typing discrepancies.

Technical causes	Incorrect matching of red cell and serum test results
	Incorrect recording of results
	Failure to add serum or red cells to test
	Failure to recognize hemolysis as a positive test result
	Improper warming of test system
	Incorrect ratio of red cells to serum
	Improper centrifugation
	Bacteriologic contamination of reagents
	Weak antigen on test red cells
	Fibrin clots
Patient and donor-related causes	Cold agglutinins
	Abnormal serum protein values
	Infusion of rouleaux-inducing agent
	Antibody against dye in test serum
	Antibody against ingredient in medium
	Antibody active at room temperature
	Weak or absent antibody due to age or disease
	Non-ABO antibodies
	Recent transfusion
	Previous stem cell transplant
	Polyagglutinable state
	Neonatal patient
	Immunodeficient patient

Thus, potentiating agents such as antihuman globulin (AHG) were necessary to demonstrate the reaction. However, this requires incubation at 37°C, washing, and addition of the AHG. To simplify the typing procedure and to make it similar to ABO typing, reagents called high protein or rapid tube have been used for Rh typing because they promoted rapid agglutination of red cells coated with anti-D without incubation and the use of AHG. The production of an ideal high-protein or rapid tube reagent for Rh typing was unique to different companies, and sera from different companies sometimes gave different reactions with a few individuals' red cells. Red cells from some individuals would react with some antisera but not others. These individuals were said to have a Du or weak D antigen (see Chapter 9). Since their red cells contain some D antigen, it is important to detect these individuals. In the past, this is done by testing all donors for the weak D if they were initially found to be Rh negative. Present antisera that are polyclonal high protein, chemically modified low protein, or a combination of polyclonal–monoclonal low-protein reagents, detect weak D red cells and so additional testing of Rh-negative red cells is no longer necessary. The presence of the weak D antigen makes the red cells possibly immunogenic and they must be considered Rh positive (see Chapter 9).

It is not customary to type donor blood for other antigens of the Rh system. Because they are less antigenic than D, the phenotype frequencies

are such that incompatibility between donor and recipient are less common and/or the antibodies are less clinically dangerous.

Red blood cell antibody detection

Blood donors who have been previously pregnant or transfused may have red cell alloantibodies in their plasma. These antibodies can cause a positive direct antiglobulin test (DAT), shortened red cell survival, or hemolysis in the recipient [4–5]. Thus, transfusion of antibody in components containing donor plasma should be avoided. Production of red cells using present methods leaves only a small amount of residual plasma (see Chapter 5). Thus, with present methods there is probably little chance that an amount of antibody large enough to cause a clinical problem would be transfused with a unit of red cells. However, plasma or platelets may also be prepared from the donor unit, and these plasma-containing components can provide passive antibody. Other reasons to search for red cell antibodies in donor plasma are (a) the presence of an antibody means that the donor's red cells are negative for the corresponding antigen, and this is a convenient way to identify antigen-negative donors, (b) the donor's plasma may be a useful source of antibody reagent, and (c) the antibody may lead to better understanding of a blood group system. Thus, units of donated blood are screened for red cell antibodies. Plasma-containing components from units found to contain antibodies are not used for transfusion. Approximately 0.5% of donor units will test positive in the antibody detection test.

Since the red cell antibodies of concern are formed in response to pregnancy or transfusion, American Association of Blood Banks (AABB) standards require antibody detection testing only of those donors [6]. However, from a practical standpoint it is more convenient for the blood bank to screen all donor units. This avoids the possibility that a unit that should be screened will be missed or that clerical errors will result in release of a unit that contains an antibody. Because the donor antibody is diluted when it is transfused, the donor antibody detection procedure may be different from that used to detect antibodies in patients. In donor antibody detection tests, serum from several donors or several reagent red cell suspensions may be pooled. This reduces the sensitivity of the test but also reduces costs and appears not to reduce safety. Pooling of both donor sera and reagent red cells is not recommended. The antibody detection method must demonstrate clinically significant antibodies, but one of several different techniques can be used to accomplish this. The techniques include incubation at 37°C with reagent red cells in one of the following media: saline, albumin, low ionic strength solution, polyethylene glycol, or polybrene. The sensitivity of these methods and the particular antibodies they detect varies (see Chapter 10), as does their desirability for donor antibody detection. Tests are not carried out at room temperature because of the large number of nonspecific or nonclinically significant cold reactive antibodies that are detected. Currently the most common donor antibody detection method uses two or occasionally three different reagent red cell suspensions and 37°C incubation of donor serum suspended in albumin

for 30 minutes followed by AHG. This is a reasonable compromise providing a high likelihood of detecting red cell antibodies and yet minimizing the likelihood of obtaining false-positive reactions. Currently gel, solid-phase systems, and affinity columns can be used for red cell antibody detection. When solid-phase tests are used [7, 8], the microtiter plates are covered with either ghosts of the reagent red cells or AHG. For plates covered with red cell ghosts, the donor's serum is added and the antibody–antigen reaction occurs on the plate surface. Antibody binding is detected by adding anti-IgG-coated red cells. For plates covered with AHG, the antibody–antigen reaction can occur in the fluid phase and the mixture added to the wells where IgG-coated red cells adhere to the AHG-coated plates. In the affinity column system, the antibody–antigen reaction can also occur in the fluid phase. AHG is added, and the reagent red cells are then added to the microwell plates. If antibody is present, the IgG–anti-IgG complex on the red cell binds to the plate coated with staphylococcal protein A. These solid-phase tests are similar in their ability to detect donor antibodies, although the titers of the antibodies differ in the different methods [8].

Automated devices designed for large-scale screening in donor centers have been developed for the solid-phase system or based on agglutination, which is read either by photometers. The instruments incorporate automated sample and reagent dispensing have bar code readers for sample identification, and use anticoagulated blood so that both red cells and plasma can be sampled from the same tube and centrifugation with sample separation is not necessary. Each blood center will establish the method that provides the most satisfactory results for their donor population and technical staff.

Positive direct antiglobulin tests in normal donors

A positive DAT occurs in about 1 per 7000–14,000 donors [9, 10]. Occasionally the positive DAT result is caused by a viral infection or immune disease, but usually there is no explanation for this positive test result when the donors are observed for long periods [9]. At normal ionic strength, about 2×10^3 molecules of IgG are bound to the red cell. The amount of IgG binding can be increased by lowering the ionic strength of the medium or treating the cells with enzymes. The positive DATs in normal donors are due to unknown factors causing increased binding of IgG to the red cells. Some reports of higher incidence of positive DATs in normal donors are probably the result of inclusion of patients with disease. For instance, three donors have been described in whom a positive DAT was due to antiphospholipid antibodies [9]. The authors speculate that the positive DAT was caused by nonspecific binding of the antibody onto phospholipids of the red cell membrane. These antiphospholipid antibodies are associated with several autoimmune disorders (including systemic lupus erythematosus, infections, or malignancy), and the antibodies may occur in apparently healthy people. These three donors also had false-positive serologic tests for syphilis, although most donors with a positive DAT do not. Antiphospholipid antibodies probably do not

account for most of the cases of positive DATs in normal donors because the three reported donors accounted for only 10% of the donors found to have a positive DAT in that study.

8.2 Testing for transmissible diseases

General concepts of testing for transmissible diseases

The use of laboratory tests to eliminate potentially infectious blood from the blood supply has been in place since the 1950s, when syphilis testing became routine. The use of testing to improve the safety of transfusion therapy is discussed more fully in Chapter 15. The decision to implement a test and the strategy used in dealing with the test results are complex issues [11]. The quality of the test, the prevalence of the disease in the donor population, and the likelihood of transmission of the disease to blood recipients are some of the important factors to consider. The impact of a test done on millions of individuals must also be considered. Tests that perform very well in a patient population have different ramifications in a normal donor population. For instance, a test with a specificity of 99.9% might be considered excellent, but still 0.1% of positive tests will be false. For instance, if this test is done on 12 million blood donors, 12,000 individuals will have a false positive test result. However, if the disease being tested for has a very low incidence, such as 1 per 500,000 in a blood donor population, only 24 people would truly have the disease in the 12 million tested. This means that even with this excellent test that would detect the 24 infected donors, 11,976 people could be falsely labeled as having the disease. Thus, the implementation of tests in the blood donor population where the true incidence of disease is very low presents some unusual and complex issues. If such a test is an important step in improving the safety of the blood supply, it should be implemented, but along with the implementation, plans must be in place to deal with the donors who will have a false-positive test result. Confirmatory testing should be available to distinguish those who are truly positive from those who are not, and effective systems must be in place to carry out the confirmatory testing and provide this information. For donors who have a true positive test, complete and accurate information must be available describing the significance of the test result.

The process of notifying donors of abnormal infectious disease testing results is sensitive and may lead to confusion or emotional distress. In general, the process appears to work effectively but some donors may not understand that they are ineligible for future donation and many others are confused or upset [12].

Testing in resource limited settings

In highly endemic and resource limited parts of the world, it may be more cost-effective to screen donors with rapid tests before collecting their blood [13]. This could be followed with various testing strategies such as beginning with hepatitis B because this is the most prevalent disease, then

Table 8.4 Present transfusion-transmitted disease screening.

Agent	Disease
Treponema	Syphilis
Hepatitis Bs antigen	Hepatitis B
Hepatitis Bc antibody	Hepatitis B
	Hepatitis non-A, non-B[a]
	HIV[a]
Hepatitis C antibody	Hepatitis C
Hepatitis C nucleic acids	Hepatitis C
HIV 1 and 2 antibody	AIDS
HIV antigen	AIDS
HIV nucleic acids	AIDS
HTLV-I antibody	Leukemia
	Lymphoma
	Tropical paresis
HTLV-II antibody	Disease unknown
CMV[b]	CMV disease

HTLV-I, human T-cell lymphotrophic virus type I; HTLV-II, human T-cell lymphotrophic virus type II.
[a]Surrogate marker for these diseases.
[b]For immunodeficient patients only.

testing nonreactives for HIV, and then nonreactives further for syphilis and hepatitis C virus (HCV) [14]. Rapid test are often used [14a–14c].

Viral testing

The tests for specific transmissible diseases are shown in Table 8.4 and the equipment and systems in Table 8.5. The viral serology tests are immunoassay and are considered a screening test. For most of the assays, if the initial test result is positive, it is repeated in duplicate. If both of the subsequent results are negative, the test is considered to be negative and the unit of blood is suitable for use. If one or both of the subsequent results are positive, the result is considered to be "repeat reactive" and the unit of blood cannot be used.

The hepatitis B core antibody test has been used as a surrogate test for non-A, non-B hepatitis and until recently was not expected to be of value in detecting hepatitis B [15], but more recent studies suggest that it may detect early hepatitis B virus (HBV) infection [16]. Alanine aminotransferase (ALT) was used as another surrogate test for non-A, non-B hepatitis, but with the discovery of the HCV and implementation of hepatitis C testing, the ALT is no longer of value and is no longer required [15]. Donor blood is not tested for hepatitis A because this form of hepatitis is rarely transmitted by transfusion (see Chapter 15).

An important aspect of the conduct of testing for transmissible diseases is the method of handling the resulting data. Transcription errors are known to occur in medical laboratories, and they certainly occur in the management of transmissible disease test results. For instance, in New

York, during a period when eight patients were exposed to HIV-positive blood, four were due to transcription errors [17] and four were due to seronegative donation. Thus, the risk from transcription error was as great as that from a window-period donation.

The window phase

A major issue in testing for transmissible diseases, especially HIV, is that several of the assays detect antibodies, and there is an interval between infection and antibody formation. During this interval, known as the "window phase," the individual is infectious but has a negative test. Test kit manufacturers devote considerable effort to designing their test kits to provide maximum sensitivity and shorten this window phase as much as possible. The introduction of methods to test for viral nucleic acids has overcome this problem (see later).

Human immunodeficiency virus testing

For the HIV antibody test, the antigens may be prepared from materials produced by recombinant DNA or from viral extracts, depending on the manufacturer. The composition of the antigenic material used in the assays is mixed to reflect the antigen regions of HIV strains from around the world in an effort to maximize the sensitivity of the assay. The HIV assays include antigens from both HIV-1 and HIV-2. Because there is greater than 50% homology between HIV-1 and HIV-2 DNA, the HIV-1 test detects about 90% of HIV-2 strains [18]. However, as HIV-2 began to appear in the United States [19], the US Food and Drug Administration (FDA) requested that HIV test kits include antigens specific for HIV-2. This strategy has been very effective because although HIV-2 is not common in the United States, there have been no cases of transfusion-transmitted HIV-2.

The specificity of a test is the probability that the test will be negative when the infection is not present. For anti-HIV, this ranges from 99.82% to 99.9994%. In widespread clinical use the HIV antibody test has generally been shown to detect HIV antibody in 98–100% of AIDS patients (sensitivity).

8.3 Confirmatory tests

When a blood donor is found to be anti-HIV positive on initial testing, the test is repeated in duplicate. If at least one of the subsequent tests is also positive, the donor unit is said to be repeatably reactive, and that unit is discarded. Units that are not positive on the repeat test (and thus not repeatably reactive) are suitable for transfusion.

For all blood samples that test repeat reactive, supplemental or confirmatory testing is done (Table 8.5). The Western blot or immunofluorescence are used for HIV and radioimmunoblot assay (RIBA) for HCV. FDA-approved confirmatory test kits are available for anti-HIV and anti-hepatitis C but not for anti-HTLV. The Western blot confirmatory test for anti-HIV tests for antibody against the following HIV proteins:

Table 8.5 Automated testing systems for infectious disease.

Company	Device	Assay	HBc	HCV	HAV	HIV-1	HIV-2	HTLF-I/HTLV-II	Chagas	WNV	CMV
Abbott	Abbott Prism	ChLIA	X	X		X	X	X	X		
Novartis	Procleix Tigris	NAT		X	X	X					X
Tecan	Genesis	NAT/EIA									
Roche	Cobas s 202	NAT	X	X		X	X			X	
Roche	Cobas amplicor	NAT	X	X		X					
Biomerieux	Vidia	EIA	X		X	X	X				X
Biomerieux	Vidas	EIA	X		X	X	X				X
Beckman Coulter	Access2	CLIA	X		X	X	X				X
Grifols	Triturus	EIA									

EIA, Enzyme Immunosorbent Assay; HBc, hepatitis B core antibody; HBV, hepatitis B virus; HCV, hepatitis C virus; NAT, Nucleic acid amplification testing.

p24, the major core (gag) protein; gp41, a transmembrane (env) protein; and gp120/160 external (env) protein and an external (env) precursor protein. The results of the Western blot can be positive, indeterminate, or negative. The criteria for positivity on the Western blot are the presence of antibody against any two of these proteins. Sera that react with only one protein are considered indeterminate. These individuals must be deferred from blood donation, although it appears that they are not infected with HIV [20, 21], and it has been proposed that they be reinstated as blood donors [22, 23]. A few individuals with an indeterminate pattern of reactivity on the Western blot may show conversion to a clearly positive pattern in blood samples obtained a few weeks later, but most do not show any change in the pattern of reactivity of their serum. False-positive screening test results for anti-HIV and anti-HTLV (not confirmed by Western blot) have been related to receipt of an influenza vaccine [24, 25]. False negative tests are mostly due to borderline reactivity, although technical and clerical errors occur [26].

8.4 Shortening the window phase

The approach to shortening the window phase is to add the test methodology to detect the infectious agent.

HIV antigen test

In an effort to detect HIV-infected donors earlier, the FDA required the addition of a test for the HIV antigen. Initial testing used an immunoassay. Two studies of several hundred thousand donors failed to identify any donor who tested positive by the HIV antigen test but negative by the anti-HIV test [27, 28]. Thus, it was considered unlikely that HIV antigen screening would be valuable. However, a few cases of transmission of HIV from antigen-positive, antibody-negative donors occurred [29, 30], and so more sensitive approaches to detect the infectious agents were sought.

Table 8.6 Sequence of testing of donated blood.

Pathogen	Test	Confirmatory Test
HIV-1, -2	HIV-1/2EIA	HIV-1 Western blot or immunofluorescence assay
		HIV-2 Western blot or immunofluorescence assay
		HIV-2 EIA
	HIV-1 NAT	Confirmatory NAT, demonstration of seroconversion on follow-up
HCV	EIA/ChLIA	RIBA or RNA test
	HCV NAT	Confirmatory NAT, demonstration of seroconversion on follow-up
HBV	HBsAg EIA/ChLIA	Neutralization test
	Anti-HBc EIA/ChLIA	No licensed test

Source: Adapted from Roback J. Technical Manual, 16th ed. Bethesda, MD: American Association of Blood Banks, 2009, p. 261.
EIA, Enzyme Immunosorbent Assay; HBc, hepatitis B core antibody; HBV, hepatitis B virus; HCV, hepatitis C virus; NAT, Nucleic acid amplification testing.

Nucleic acid amplification testing

At an FDA-sponsored conference in 1995, the Commissioner asked industry and the blood bank community to develop methods using nucleic acid amplification to detect the viral genome [31]. The manufacturers were skeptical but under pressure estimated that such assays could be available by late 1998–2000. The methodology was developed with unprecedented speed, and the FDA developed policies that allowed nearly all blood to be tested while the methods were still in clinical trial [32, 33]. Donor blood is tested in pools of about 8, which makes the nucleic acid amplification testing (NAT) technology somewhat practical. The pool testing detects about 5 viral copies per milliliter that equals about 80–7200 copies per milliliter of donated blood [32, 33]. Although NAT testing, at least in Europe, has identified fewer donors than expected [34], infected seronegative donors have been found and transfusion of their infectious blood has been avoided [34–38]. All donated blood and components are now tested by NAT for HIV and HCV.

8.5 Human T-cell lymphotrophic virus

Donors are tested for antibodies to human T-cell lymphotrophic virus type I (HTLV-I) and human T-cell lymphotrophic virus type II (HTLV-II) using an Enzyme Immunosorbent Assay (EIA) method [39]. The assays from different manufacturers have varying capability to detect HTLV-II. The testing algorithm is the same as that described for HIV. Supplemental confirmatory testing is also done using a Western blot with recombinant DNA-produced and viral lysate antigens.

8.6 Hepatitis tests

Hepatitis B surface antigen

Testing for hepatitis B was introduced in 1971 shortly after the discovery of the hepatitis-associated antigen. Test methodology evolved over the years, and at present an immunoassay system is used. Antibody to hepatitis B surface antigen (HBsAg) is coated onto particles, the donor's serum is added, and any HBsAg binds to the particle and is detected by a second anti-HBs that is linked to an enzyme. The HBsAg test is confirmed using a neutralization step. This is done by adding known anti-HBs and repeating the assay. A substantial reduction in activity signifies a true positive or confirmed positive test.

Hepatitis B core antibody

The hepatitis B core antibody (anti-HBc) test was originally introduced in hopes of identifying cases of hepatitis B in a phase with no detectable HBsAg [40]. It appears that anti-HBc does detect a few donors infectious for hepatitis B but with a negative HBsAg test [15, 16, 40–43]. The anti-HBc test is different than the others in that it is an inhibition-type assay. HBc is bound to the solid phase, and the indicator (enzyme-linked) probe is anti-HBc. The anti-HBc in the probe competes with the donor's serum sample, and if anti-HBc is present in the donor's serum there is less binding of the labeled probe and a reduced assay activity, indicating a positive test. Unfortunately, this type of assay is subject to greater variability than the direct-type assay used for other tests, and this has resulted in a considerable false-positive rate for the anti-HBc test.

NAT for hepatitis B

NAT has not been introduced for hepatitis B because the slow doubling time of that virus causes low levels of viremia during the window phase [33]. HBV DNA levels in HbsAg-positive and anti-HBc-positive blood donors can be extremely low. Current mini pool HBV-NAT methods would miss about 6% of these donations and about 3% would not be detected even by single donor NAT. Therefore, the widespread adoption of NAT testing for HBV has not occurred [44]. It is still hoped that improved immunoassays will avoid the need for NAT for hepatitis B [33].

Hepatitis C antibody

The hepatitis C antibody (anti-HCV) test is also an immunoassay. Peptides of the HCV are bound to the solid phase, and an enzyme-linked antiglobulin is the detection system. The mix of peptides in the assay system has been modified over the years improving the test performance and reducing the proportion of false-positive reactions [45]. A confirmatory test is available using an immunoblot system [46].

Blood donors who have confirmed NAT positive results for HCV in the absence of anti-HCV are likely to have been recently infected. The most common reason for this is injection drug use [47]. However, even

immunocompetent individuals may not have a persistent HCV antibody response [48].

Surrogate testing for hepatitis using ALT

Studies during the 1970s and 1980s indicated that blood from donors with an elevated ALT was associated with transmission of non-A, non-B hepatitis [40, 43] (see Chapter 15). In response to these data, many blood banks initiated testing for both anti-HBc and ALT. Several difficulties were found in using ALT as a donor screening test. The values are influenced by age, gender, race, and weight, and the results are a continuous distribution, making determination of a "cutoff" difficult. Nevertheless, the use of ALT prior to introduction of HCV testing seems to have been helpful in reducing posttransfusion non-A, non-B hepatitis (see Chapter 15). However, with the availability of the specific test for anti-HCV, the ALT no longer contributes to blood safety, and it has been discontinued [15].

8.7 Managing the results of transmissible disease testing of donors

Donors must be notified of the results if a screening test is positive. A repeated reactive (positive) screening test result may or may not subsequently be confirmed. Thus, the information provided must be tailored to the specific disease and the nature of the test result. For instance, even a nonconfirmed but positive initial screening test for HIV may create serious anxiety in the donor. The information provided should describe the disease, the significance of the test result, and any recommendations or implications this has for the donor's health and future blood donations. The extent to which blood bank personnel should take responsibility for providing this information is an interesting issue. Blood bank personnel usually take primary responsibility for at least the initial consultation. This is particularly important in notifying people of a positive HIV test. Since the ramifications of this result are so serious, it is important to do this personally and to have information and referral systems to knowledgeable AIDS-treating physicians readily available. Another complicating matter is that the significance of the test result may not be known. For instance, the long-term clinical effect of hepatitis C has only become clear during the last few years. During the early stages of hepatitis C screening of donors, there was no licensed confirmatory test, and as a result a large portion of the donors who had a positive screening test were probably not infected. Thus, the donors had to be told they had a positive screening test for hepatitis C, but it was not known whether they were infected. Needless to say, this created considerable anxiety. Another example is the lack of definitive information on the long-term health impact of an indeterminate Western blot for HIV. While it appears that these donors may not be infected (see above), they are deferred from donating blood and cannot be told definitively that despite a positive screening test they are not infected.

8.8 Lookback

When a donor is found to have a positive test for HIV, HTLV, hepatitis C, or hepatitis B, records are reviewed to determine whether the individual has donated previously. If so, the hospital to which the blood was provided is notified. They in turn notify the physician of the blood recipient, who is expected to determine whether and how to inform the patient. This process is called lookback. The objectives of lookback are to alert the patient so that he or she can obtain treatment for the disease if indicated and take steps to avoid transmitting the disease to others. Lookback for hepatitis C has been complicated by the multiple versions of the screening immunoassay and confirmatory immunoblot with different sensitivities and specificities that have been used at different times. The FDA guidance on lookback was designed to minimize the number of notifications of recipients of potentially false positive donor blood while identifying recipients who might have received infectious blood [49, 50]. Hepatitis C lookback was successful in identifying some HCV-infected patients who were unaware of their status [51–54], but in the United States only 1–2% of transfusion recipients were unaware of this [51]. The overall value of hepatitis C lookback as a public health strategy has been questioned [51].

8.9 Syphilis testing of donated blood

Syphilis testing has been carried out on donated blood for more than 50 years because of the recognized risk of transfusion-transmitted syphilis [55] (see also Chapter 15). However, very few cases have been reported for years, and in the mid-1980s a move was initiated to discontinue testing. This was because the spirochete is viable in stored blood for only about 96 hours [15], and the serologic test for syphilis is rarely positive at a time when spirochetes are in the donor's circulation. A recent study [56] using nucleic acid amplification technology did not find treponema pallidum DNA or RNA in any of 169 confirmed STS-positive samples from blood donors. Thus, the current testing probably does not identify infectious units [55–57]. However, as the AIDS epidemic began to unfold, the syphilis test was retained as a surrogate marker of risk for sexually transmitted diseases, including HIV. This has not proved to be an effective way to identify HIV-infected donors [15], and syphilis testing need not be retained for this purpose. Despite the fact that many blood components, especially platelets, are stored at room temperature conditions that will not inactivate the spirochete, Schmidt pointed out that there are many reasons to discontinue syphilis testing in the United States [57], but Greenwalt did not completely concur [58]. Syphilis is a more common disease in many parts of the world and testing makes more sense there, but the problem still remains that most screening methods are not positive when there are circulating spirochetes. The issue of syphilis testing seems to have little

scientific basis but is caught up in the FDA's need to be aggressive in blood safety policies.

8.10 West Nile virus

West Nile virus (WNV) infection can be transmitted by transfusion (see Chapter 15). A nucleic acid amplification test was developed with unprecedented speed partly due to existing NAT technology for HIV and HCV testing. Test of blood donated from epidemic areas began in early summer 2003 [59–62]. While a few infectious units may not be detected due to low levels of viremia [63], NAT testing has been quite effective in reducing the likelihood of transfusion-transmitted WNV infection [59–62]. Routine testing is done in pools similar to HIV and HCV, but due to low-level viremia, WNV is switched to individual testing during epidemic season. WNV testing is a nice example of the public health aspects of blood banking in that test results in the donor population can provide valuable information about community-wide disease prevalence [64].

8.11 Chagas' disease

Trypanosoma cruzi, a parasite, can also be transmitted by transfusion (Chapter 15). Many patients with chronic T. cruzi infection may be asymptomatic and thus could pass the blood donor medical questions [65]. The organisms can survive in refrigerated blood and can be transmitted by transfusion [66], although cases of transfusion-transmitted Chagas' disease are extremely rate in the United States [67, 68]. Antibody tests are currently being evaluated [69].

8.12 Bacterial detection

It has been known for years that a small percentage of units of whole blood contain viable bacteria [70]. There are two general types of concerns: bacterial contamination of platelet concentrates stored at room temperature and transmission of bacteria, especially *Yersinia enterocolitica*, from red cells stored at refrigerator temperatures. For either of these situations, the sources of potential contamination are usually blood or the skin at the venipuncture site, and rarely the environment such as air, equipment, water, and the phlebotomist and rarely the blood collection pack. Steps taken to minimize the possibility of transfusion-associated sepsis include effective skin decontamination, diversion of the first 20–30 mL of blood (see Chapter 4), proper storage and handling conditions, and detection of bacteria before transfusion [70, 71] (see Table 14.10).

The method widely used at this writing is the culture systems that are used for patient blood cultures [72]. The BacT/ALERT system can be used

for the detection of bacteria in platelet products with the ability to detect levels of 10 and 100 CFUs per milliliter [73].

Drawbacks to this approach are that the sample is taken after several hours to provide time for bacteria to proliferate and reach a detectable level and then the culture requires about 18 hours. Thus, the testing usually involves a 1-day delay in product release. In addition, culturing individual units of whole-blood-derived platelets is expensive and this has led to further decline in their use. Another system tests platelets at the release for transfusion when the level of organisms is higher and thus more easily detected [74]. Current bacterial detection systems do not detect all contaminated units and they have not eliminated transfusion-related septic fatalities [75–77].

8.13 Optional tests of donor blood

Cytomegalovirus

Cytomegalovirus (CMV) infection can be transmitted by blood transfusion, resulting in serious, even fatal, disease (see Chapters 11, 12, and 15). The primary CMV infection can be mild or asymptomatic, and the CMV then remains latent in healthy individuals, whose blood can transmit the disease to susceptible patients [78, 79]. It is thought that the virus remains latent in the donor's leukocytes, but efforts to develop a test to detect the virus and thus define true infectivity have not been successful. As a result, the testing strategy has had to identify potentially infectious donors by detecting previous exposure to the virus. This is done using a test for CMV antibody. Alternatively, CMV transmission can be prevented by removing the leukocytes from the blood components (see Chapter 11). This section deals with screening donated blood for anti-CMV to prevent CMV transmission.

CMV testing is not done routinely on all donated blood. Several types of CMV antibody detection methods are available, including enzyme-linked assays (EIA), latex agglutination, solid-phase immunofluorescence, complement fixation, and indirect hemagglutination. These methods can be used to detect IgG or IgM antibodies with a specificity of 85–98% and a sensitivity of 95–99% [80]. The two assay methods used most commonly are the EIA and the latex agglutination test. The EIA is done as described above for other transfusion-transmitted diseases. The microtiter plates are coated with CMV antigen; the patient's serum is added, incubated, and washed away; and any antibody in the patient's serum bound to the plate is detected with the enzyme-linked antibody. The latex agglutination test uses latex particles coated with CMV antigen. Antibodies, if present in the patient's serum, bind to the latex particles and the particles form macroscopically visible agglutinates. The latex test is more convenient to carry out if only a few samples are to be tested, and this test tends to be used in hospitals to screen units of blood in their inventory. The EIA method lends itself better to large-scale testing. Also, since the EIA test is similar to other infectious disease tests, it tends to be the test method of

choice in blood centers for screening large numbers of donors. Both tests are done on serum or plasma.

CMV infection is rather common in the general population, with 30–80% of blood donors testing positive with the EIA or latex tests; but probably only about 1–3% of donors are actually infectious [80]. Unfortunately, these antibody tests do not distinguish those donors who are truly infectious. IgM antibodies may indicate recent infection and suggest that a donor is more likely to be infectious, but this has not proved to be very effective in identifying truly infectious donors. Although polymerase chain reaction is a very sensitive assay to detect viral DNA, this has not yet been developed into a practical screening test for CMV in blood donors. Thus, the donor-screening approach to prevention of transmission of CMV is screening of donors for antibody to CMV and use of only CMV antibody-negative blood components for susceptible patients.

Parvovirus and hepatitis A virus

These viruses can be transmitted by transfusion (see Chapter 15) but the main concern with these viruses is transmission via plasma derivatives, since the viruses are not inactivated during manufacture. NAT techniques are available and effective for detection of parvovirus and hepatitis A virus (HAV) and are often discussed together because of regulatory issues, not biological similarity. NAT for these viruses is now done for source plasma (intended for the manufacture of derivatives). NAT for parvovirus and HAV is presently considered an "in-process control" by FDA and, thus, donor notification is not required. If NAT for parvovirus and HAV is used for whole blood donors, the regulatory framework of in-process control versus donor screening test with its attendant ramifications will need to be determined.

Other red cell antigens

Donors are not routinely typed for other red cell antigens. Occasionally a blood bank or donor center may wish to screen donors for particular antigens to increase the blood center's rare donor file. The particular antigens being sought depend on local circumstances and the availability of good reagents.

HLA or granulocyte antibody detection in donors

Because of the possibility that HLA or granulocyte antibodies in donors may be involved in transfusion-related acute lung injury (TRALI) (see Chapter 14), it has been suggested that blood from multiparous donors not be used as whole blood or plasma components unless it has been screened for these antibodies [81]. This practice has not been implemented, possibly because newer information suggests that cytokines or other inciting factors may play an important role in TRALI and screening of donors for these antibodies may not be especially helpful. This remains an important issue, however, and such screening may be implemented in the future so that transfusion of plasma containing these antibodies can be avoided. An

alternative to laboratory testing is to avoid using plasma from multiparous or previously transfused donors.

Platelet-specific antibodies and antigens

Donors' blood is not routinely tested for platelet-specific antibodies. Occasionally some blood centers may screen donors for a limited time to identify donors negative for the HPA1 (PlA1) platelet-specific antigen. This is the antigen most commonly involved in alloimmune neonatal thrombocytopenia or posttransfusion purpura (see Chapters 10 and 12), and many blood centers like to maintain a file of donors known to lack this antigen. The donors can be called upon to donate platelets if needed for patients with anti-HPA1 antibodies.

Screening donors for IgA deficiency

IgA levels are not routinely determined in donated blood. Some blood centers occasionally screen donors for a limited time to establish a file of donors known to be IgA deficient. The donor record can be annotated so that when the individual donates, the plasma from the unit can be frozen and saved for future use for IgA-deficient patients. Also, those donors can be called upon to donate platelets if needed for IgA-deficient patients.

8.14 Summary

Laboratory testing of donated blood is composed of both red cell typing and transmissible disease testing. Each of these kinds of tests is under increasing stringency. As electronic systems are used increasingly for data management, repeated red cell laboratory tests are being eliminated, placing increased importance on the accuracy of the original donor type result (see Chapter 10). Transmissible disease tests are an essential part of the strategy to minimize the risks of blood transfusion. The increasing complexity of these tests has led to consolidation of the testing into larger laboratories and the use of computer control systems, making the entire donor testing process much more extensive and intricate than just a decade ago.

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9 Blood Groups

RBC cell surface antigens are classified by the International Society of Blood Transfusion. A total of 308 red cell antigens are classified as systems, collections, low-frequency antigens, and high-frequency antigens [1, 2]. Of these, 270 are categorized in 30 major discrete systems (Table 9.1). This chapter describes the red cell antigens and systems and the platelet and neutrophil antigens and antibodies that are most commonly involved in the clinical practice of transfusion medicine.

The biochemical composition, molecular weight, and number of antigens per red cell have been established for many antigens (Table 9.2). The nature of the molecules and the genes responsible have been identified for all of the 30 blood group systems and the function of many of these molecules is known [3, 4]. Reference texts [4, 5] provide a more comprehensive description of the hundreds of known red cell antigens. An outstanding review of the red cell membrane was published in 2008 [6]

There are two basic kinds of blood group antigens. Carbohydrates attached to either proteins or lipids determine one group of antigens. The specificity of these blood group antigens is determined by sugars, and thus the genes responsible for these antigens code for an intermediate molecule, usually an enzyme that creates the antigenic specificity by transferring sugar molecules onto the protein or lipid. Thus, certain proteins exposed on the outer surface of the red cell will express the carbohydrate-determined antigens (Figure 9.1). The carbohydrate-defined antigens are ABO, Lewis, Hh, and P. The second type of antigen is determined by amino acid sequences of proteins that are directly determined by genes. It is believed that proteins carrying blood group antigens are inserted into the red cell membrane in one of three ways: single pass, multiple pass, and linked to phosphatidylinositol (Figure 9.2). The size of the molecule containing the blood group antigens ranges from 25 to 100 kD, and the number of antigen sites from just 1500 to more than 1,000,000 per red cell (Table 9.2). The following discussion provides a brief synopsis of the major blood group systems.

Table 9.1 Major red blood cell group systems containing 230 antigen specificities.

Number	Name	Symbol	Number of antigens
001	ABO	ABO	4
002	MNS	MNS	46
003	P	P1	1
004	Rh	RH	50
005	Lutheran	LU	19
006	Kell	KEL	31
007	Lewis	LE	6
008	Duffy	FY	6
009	Kidd	JK	3
010	Diego	DI	21
011	Yt	YT	2
012	Xg	XG	2
013	Scianna	Sc	7
014	Dombrock	DO	6
015	Colton	CO	3
016	Landsteiner-Wiener	LW	3
017	Chido/Rogers	CH/RG	9
018	H	H	1
019	Kx	XK	1
020	Gerbich	GE	8
021	Cromer	CROM	15
022	Knops	KN	9
023	Indian	4	4
024	Ok	OK	1
025	Raph	RAPH	1
026	John Milton Hagen	JMH	5
027	I	I	1
028	Globoside	GLOB	1
029	Gill	GIL	1
030	RHAG	RHAG	3

Source: Garratty G. Advances in red blood cell immunology 1960 to 2009. Transfusion 2010; 50:526–535. Daniels G, Castilho L, Flegel WA, et al. International Society of Blood Transfusion Committee on Terminology for Red Cell Surface Antigens: Macao Report. 3. Vox Sang 2009; 96:153–156. Daniels G. Functions of red cell surface proteins. Vox Sang 2007; 93:331–340. Daniels G. Human blood Groups, 2nd ed. Cambridge, MA: Blackwell Science, 2002. Reid ME, Lomas-Francis C. The Blood Group Antigen Facts Book, 2nd ed. London: Academic Press, 2004. Daniels GL, Fletcher A, Garratty G, et al. Blood group terminology 2004. From the ISBT Committee on Terminology for Red Cell Surface Antigens. Vox Sang 2004; 87:304–316.

9.2 ABO system

The ABO system was the first red cell blood group system to be identified, and its discovery led to the Nobel prize for Landsteiner (see Chapter 1) [7]. Landsteiner observed agglutination reactions by mixing various

Table 9.2 Molecular and biochemical characteristics of the ISBT designated systems.

Number	System name	Chromo-some of gene location	Number of antigen sites x 10^3 red cell	Molecular weight of antigen (kD)	RBC membrane components associated with antigen expression	Antigen composition
001	AB	9	1000	90–100	Anion transport protein (band 3)	Glycoprotein
018	H	19			CD 173	Glycoprotein
007	Lewis	19	Not applicable	Not applicable	Not applicable	Carbohydrate
003	P	22	Unknown	Unknown	Globoside I	Carbohydrate
004	Rh	1	100–200	30–32	Polypeptides	Lipoprotein
			100–200	45–100	Polypeptides	Lipoprotein
016	LW	19	3–5	40	CD 242, ICAM, IqSF	Glycoprotein
002	MNSs	4	200–1000	43[a]	Glycophorin A and B	
006	Kell	7	3–6	93[a]	CD 239 Endopeptidase	Glycoprotein
019	Kx	X	Unknown	32	Unknown	Protein
008	Duffy	1	12	40–66	CD 234 Receptor	Glycoprotein
005	Lutheran	19	1–4	78–85	Cd 239 IgSF	Glycoprotein
020	Gerbich	2	60–120	39[a]	Glycophorin C	Glycoprotein
			15–20	30[a]	Glycophorin D	Glycoprotein
009	Kidd	18	14	43	Urea transporter	Glycoprotein
012	Xg	X	Unknown	22–29	CD 99	Glycoprotein
015	Colton	7	120–160	40–60	Aquaporin	Glycoprotein
017	ChidoRogers	6	Unknown	96	C4A, C4B	Glycoprotein
026	John Multon Hagen	15	Unknown	Unknown	CDW108 semiphorin	
025	Raph	11	Unknown	Unknown	Unknown	Glycoprotein
024	Ok	19	Unknown	Unknown	CD147	Glycoprotein
010	Diego	17	15	Unknown	Band 3, Ion exchanger	Glycoprotein
011	Yt	7	Unknown	72–160	Acetylcholinesterase	Glycoprotein
021	Cromer	1	Unknown	70	Decay accelerating factor	Glycoprotein
014	Dombrock	12	Unknown	46–57	Rebosyl transferase	Glycoprotein
013	Scianna	1	Unknown	60–68	Unknown	Glycoprotein
022	Knops	1	Unknown	200	CD35; CR1	Glycoprotein
023	Indian	11	6–10	80	CD44	Glycoprotein
	Ii	Unknown	120	55	Glucose transport protein (band 4.5) Poly-*N*-acetyl lactosaminyl glycolips	Glycoprotein
						Glycolipid

Source: Adapted from Garratty G. Advances in red blood cell immunology 1960 to 2009. Transfusion 2010; 50:526–535. Daniels G, Castilho L, Flegel WA, et al. International Society of Blood Transfusion Committee on Terminology for Red Cell Surface Antigens: Macao Report. 3. Vox Sang 2009; 96:153–156. Daniels G. Functions of red cell surface proteins. Vox Sang 2007; 93:331–340. Daniels G. Human blood Groups, 2nd ed. Cambridge, MA: Blackwell Science, 2002. Reid ME, Lomas-Francis C. The Blood Group Antigen Facts Book, 2nd ed. London: Academic Press, 2004.
[a]These molecules give anomalous apparent molecular weight on SDS PAGE. Fy antigen activity is observed over a broad range of molecular weights (38–90 kD); the value given corresponds to the region of greatest activity.
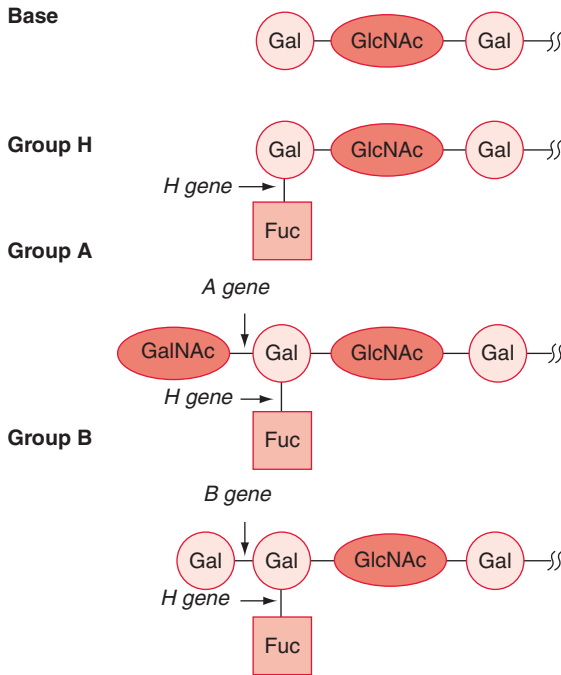
Figure 9.1 Schematic diagram of the site of action of ABH genes. Gal, galactose; Fuc, fucose; GlcNAc, *N*-acetyl glucosamine; GalNAC, *N*-acetyl galactosamine.

combinations of cells and sera from himself, four doctoral students working in his laboratory, and another subject. This simple set of experiments was the beginning of red cell serology and blood groups. Landsteiner had identified the clinically most important red cell antigen system (Table 9.3), because most individuals have circulating A and B antibodies that are usually complement-fixing antibodies and cause intravascular hemolysis. The antigenic determinants for A, B, and H are found widely throughout our environment on bacteria, plants, food, and dust. Exposure to these antigens causes the normal development of antibodies against whichever ABH antigens are absent in the individual (Table 9.4).

Genes and composition

ABH antigen activity is determined by sugars that are linked either to polypeptides (forming a glycoprotein) or to lipids (forming a glycolipid). Because the antigenic activity is determined by sugars in a carbohydrate structure, the antigens are not directly determined by genes. Instead, the ABH genes determine proteins that are sugar-transferring enzymes collectively called glycosyltransferases. Genes for three different blood group systems (ABO, Hh, and Sese) control the expression of ABO antigens. Each A, B, and H gene codes for a different enzyme (glycosyl
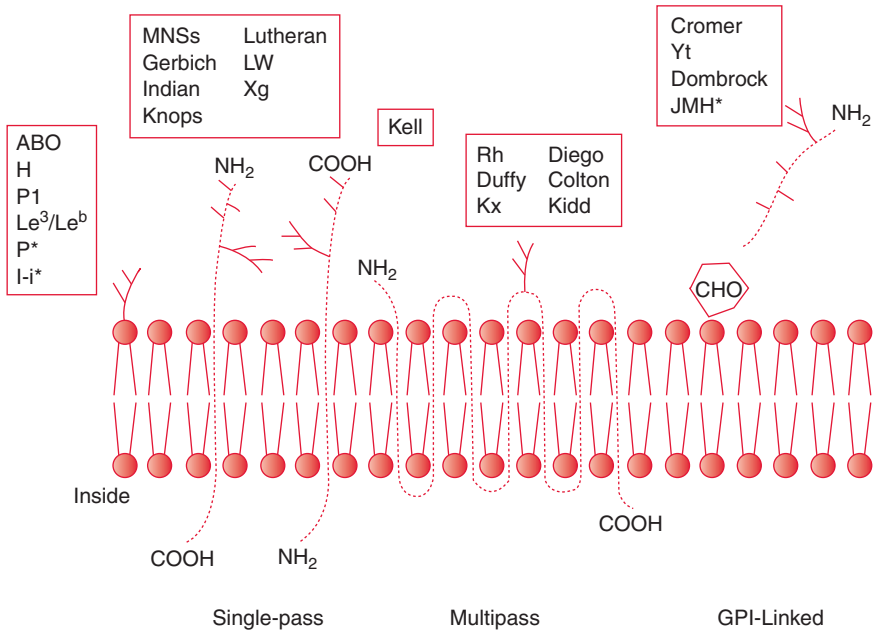
Figure 9.2 Models for insertion of RBC membrane proteins. The blood group systems that are associated with different RBC membrane components are shown in the boxes. An asterisk represents a blood group collection or unassigned high-prevalence antigen. CHO represents carbohydrate moieties. The multipass membrane proteins carrying Rh, Kidd, Diego, Colton, and Kx antigens are oriented with their amino (NH_2) and carboxyl (COOH) termini to the inside of the membrane. The number of membrane passes differs for each protein, as does the glycosylation. (Reproduced with permission from Reid ME. Molecular basis for blood groups and function of carrier proteins. In: Silbertsein LE, ed. Molecular and Functional Aspects of Blood Group Antigens. Bethesda, MD: American Association of Blood Banks, 1995:75–126.)

Table 9.3 A_1A_2BO phenotype, gene and genotype frequencies in southern England in 1939.

Phenotype	Frequency	Gene	Calculated frequency	Genotype	Calculated frequency
O	0.43	O	0.6602	O/O	0.43
A^1	0.35	A^1	0.2090	A^1/A^1	0.04
				A^1/O	0.28
				A^1/A^2	0.03
A^2	0.10	A^2	0.0696	A^2/A^2	0.00
				A^2/O	0.09
B	0.09	B	0.0612	B/B	0.00
				B/O	0.08
A_1B	0.03			A^1/B	0.03
A_2B	0.01			A^2/B	0.01
	1.00		1.0000		1.00

Source: Adapted from Daniels G. Functions of red cell surface proteins. Vox Sang 2007; 93:331–340.

Table 9.4 Genes and antigens of the ABH system and ABO type of donor red cells suitable for patients of different ABO types.

Gene	Glycosyl transferase	Antigen-determining sugar	ABN antigen	ABO type	Antibody present	Preferable donor	Other acceptable donors[a]
A	*N*-Acetyl galactosamine transferase	*N*-Acetyl galac-tosamine	A	A	B	A	O
B	D-Galactosyl transferase	D-Galactose	B	B	A	B	O
H	L-Fucosyl transferase	L-Fucose	H	O	A & B	O	None
A & B	All above	All above	A & B	AB	None	AB	A or B or O[b]

[a]If blood is used as red blood cells.
[b]Group A is preferable.

transferase), which places a different sugar on the polypeptide or lipid to produce the unique antigen (Table 9.4, Figure 9.1). The genes for the ABO system are on chromosome 9 [8,9]. The O gene is similar to the A gene except for one base deletion that causes a frame shift and premature stop in transcription [10–12]. The result is lack of production of a functional protein (enzyme), and so there is no product of the O gene and O is a null phenotype of A and B. The B gene differs from the A gene by four amino acid substitutions [13] Some of the differences have more impact on the resulting protein, but in general, the substitutions result in the two different enzymes and different sugar attachments, giving different antigen specificities [12–15].

At least 40 different alleles of the O gene have been characterized but in studies of individuals from four continents (Africa, Europe, South America, and Asia), it could be determined that most O alleles are derived by point mutations from the two worldwide distributed alleles 001 and 002. Phylogenetic analysis suggests that at the evolutionary rate, the alleles of the three human ABO lineages probably developed 4.5 to 6 million years ago [16].

9.3 A and B subgroups

Before the structure and number of A and B antigens was known, it was observed that red cells from some group A individuals react weakly or not at all with anti-A sera. These were called A subgroups and were named A2, intermediate (A int), A3, Ax, Am, Ay, Ael, and Aend. It was thought that different forms of the A antigen might exist and that sera contained mixtures of antibodies to these different A subgroup antigens. Now it appears that these differences in reactivity are caused by variability in the ABO genes, resulting in variations in the A antigen structure or the number of antigen sites [3,13]. For instance, the number of A sites ranges from

approximately 800,000/red cell for A1 to 250,000 for A2 to as few as 700 for Am. In addition, the A2 gene differs from the A1 gene by one base pair, resulting in a different protein product but also differences in the number of antigen sites [3, 13]. Some individuals with these weak A subgroups form anti-A antibodies, probably indicating a qualitative difference in their A antigens. However, A2 red cells will absorb anti-A1, suggesting that the differences may be more quantitative. Thus, it appears that A subgroups may result from either qualitative or quantitative differences.

ABO variants such as A_2, A_x, etc. may be due to single nucleotide mutations [13]. Genes for the Hh system are on chromosome 19. The H gene enables the attachment of L-fucose to the polypeptide or lipid chain. If no A or B gene is present, the H specificity remains and the individual is a type O, since the O gene is nonfunctional. These group O red cells do not react with anti-A or anti-B. If an A or B gene or both are present, additional sugars are attached to the L-fucose, giving A, B, or AB specificity. Thus, ABO specificity depends on both the ABO and the Hh genes (Figure 9.1).

Bombay type

The very rare Bombay phenotype, first recognized in India, lacks the H gene and is homozygous for its allele h (they are hh). The notation Oh is used for the Bombay type. The lack of an H gene does not allow the attachment of L-fucose to the protein or lipid, and thus the individuals express very little H antigen (Figure 9.1). They type as group O, since their red cells do not react with anti-A or anti-B and their serum contains anti-A and anti-B. However, their serum also contains anti-H, which reacts with group O red cells and can cause hemolysis in vivo. It is fortunate that such individuals are rare, because finding compatible red cells is extremely difficult. The only suitable red cells are those from another Bombay individual.

Antigen distribution and subgroups

ABH antigens are widely distributed throughout the body. The ABH antigens present on red cells may be in either the glycoprotein or the glycolipid form. ABH glycolipids are also part of most endothelial and epithelial membranes. In addition, the ABH glycolipids are present in a soluble form in plasma. Most other body fluids and secretions contain soluble ABH antigens, but those are in the glycoprotein form. The ability to secrete soluble ABH antigens is controlled by a secretor (Se) gene, which is separate from the ABH system.

In a normal adult, the number of A antigen sites ranges from 1,600,000 (A1 individual) to 800,000 (A2 individual), to 700 (Am individual). The number of B antigen sites is 600,000–800,000, and the number of A, B antigen sites about 800,000 [3, 4]. Normal newborns have approximately one-third the adult numbers of antigen sites.

Antibodies of the ABH system

These antibodies begin to appear during the first few months of life, probably from exposure to ABH antigen-like substances in the environment. Thus, these antibodies are called "naturally occurring," but

the implication that they form without antigenic stimulation is incorrect. Anti-A or anti-B antibodies are usually combinations of IgM and IgG. Because of the IgM content, these sera almost always cause agglutination—even at room temperature—of red cells containing the corresponding antigen. The IgM composition makes these antibodies effective in fixing complement, and so they can be very dangerous clinically.

Immunization to A or B antigens can also occur by transfusion of incompatible red cells, inoculation with vaccines containing A or B antigens, transfusion of plasma containing soluble A or B antigens, or pregnancy with an ABO-incompatible fetus. Following these kinds of immunization, the A or B antibody may become more active at 37°C, have a higher IgG component, increase in titer and/or avidity, and become more strongly hemolytic and thus even more clinically dangerous.

The A subgroups are not of major clinical importance but may cause difficulties in the serology laboratory. Approximately 1–8% of an A individual's and 20–35% of an AB subgroup individual's sera may contain A antibodies. However, these usually do not react at 37°C and are not considered clinically significant. In rare situations where the anti-A reacts at 37°C, donors whose red cells are A2 can be easily identified, and red cells from a compatible donor can be provided. Subgroups of B exist but are rare [3]. These individuals may also have anti-B in their serum. Since donors with B subgroup red cells are also rare, group O red cells can be used for transfusion.

Anti-H antibodies can occur in persons with little or no H antigen on their red cells. Thus, Bombay type individuals (who lack the H gene and do not make H antigens) have a very active anti-H, which binds complement and causes hemolysis. A few persons of type A or AB make anti-H, probably because almost all their H antigen has been converted to A or B. These anti-H antibodies usually do not react at body temperatures and do not cause hemolysis.

9.4 The Rh system

The second most important red cell antigen system is the Rh system. This is because a substantial proportion of the Caucasian population lacks the major Rh antigen known as D; the likelihood of becoming immunized to the D antigen is very high and anti-D has serious clinical effects, causing hemolytic disease of the newborn (HDN) and/or transfusion reactions.

Discovery

In 1939, Levine and Stetson [17] reported the case of a woman who delivered a fetus affected by HDN and upon receiving a transfusion of her husband's blood experienced a severe transfusion reaction. They hypothesized correctly that the woman had become immunized to a factor that her child had inherited from the husband (father) but that she lacked. In 1940, Landsteiner and Wiener [18] obtained an antibody from guinea pigs and rabbits immunized with rhesus monkey red cells. This serum had

a pattern of reactivity similar to that of Levine and Stetson's patient, and so the blood group system was named Rh for rhesus monkey. Later work established that the animal anti-rhesus sera and the human antibody did not detect the same antigen, but by that time the nomenclature was established. The pattern of reactivity and the antigen identified by the monkey serum has been named LW in honor of Landsteiner and Wiener. LW is assigned to a different red cell antigen system.

Nomenclature and genetics

The Rh system contains at least 50 different antigens and thus is the most complex blood group system. The antigen detected in the original Levine and Stetson patient's serum is the D antigen. This is present in about 85% of North American Caucasians and is the basis for determining Rh positivity or negativity, that is, persons whose red cells contain the D antigen are Rh positive and those whose red cells lack the D antigen are Rh negative.

Four other antigens (C, c, E, and e) were identified that seemed to be inherited in various combinations along with D and became part of the Rh system (Table 9.5). These four antigens, along with D, account for almost all of the Rh-related transfusion problems encountered in practice. Different nomenclature systems have been used to describe the Rh system (Table 9.5). The Wiener system supposed that the gene product was a single entity with multiple serologic specificities [19]. The Fisher–Race system postulated three closely linked loci, each with its own gene and gene product [20]. The terms that each of these nomenclature systems applied to the different patterns of serologic reactivity are shown in Table 9.5. The Fisher–Race notations are most commonly used because they fit most easily with the serologic reactions obtained in practice. There are two Rh genes—*RHD* and *RHCE* [21]. *RHD* accounts for the many epitopes of D. There may be one or two copies of the *RHD* gene. Rh-negative individuals lack the *RHD* gene and thus that polypeptide. This explains why the d antigen was never found. The *RHCE* gene codes both the Cc and Ee polypeptides. There are four alleles at this locus: *RHCE*, *RHCe*, *RHcE*, and *RHce*. The full-length transcript of each gene encodes a polypeptide of 416 amino acids [21]. Thus, it appears that there are two Rh genes, not either one or three. Based on present understanding of the biochemical composition of the Rh antigens and their genetic control, the longstanding debate between Wiener and Race probably ends in a draw—a fitting conclusion.

Structure and composition of the D antigen

The D antigen is a 30-kD polypeptide that is associated with the red cell membrane skeleton [22, 23]. The protein has an external and intramembranous domain but no cytoplasmic domain (Figure 9.2). There is considerable similarity among the Rh proteins. The C and c antigens differ in only four amino acids, and E and e in only one amino acid. In contrast, the membrane protein in D-positive individuals possess 36 amino acids lacking in D-negative individuals. There are approximately 30,000 D antigen sites per cell [24]. These antigens cannot move laterally within the membrane but instead are fixed about 70 nm apart [25]. This distance

Table 9.5 Serologic reactions and the Weiner and Fisher–Race nomenclature for the Rh system.

Reaction with anti-					Weiner			Fisher–Race		
					Most likely genotype	Phenotype	Antigens	Most likely genotype	Phenotype	Antigens
D	C	E	c	e						
+	+	O	+	+	R^1r	R_1r hr′ hr″	Rh_0 rh′	CDe/cde	CcDee	CDce
+	+	O	O	+	R^1R^1	R_1R_1 hr″	Rh_0 rh′	CDe/Cde	CCDee	CDe
+	O	+	+	+	R^2r	R_2r rh′ hr″	Rh_0 rh′	cDE/cde	ccDEe	cDEe
+	O	+	+	O	R^2R^2	R_2R_2 hr″	Rh_0 rh′	cDE/cDE	ccDEE	cDE
+	+	+	+	+	R^1R^2	R_1R_2 rh″ hr′ hr″	Rh_0 rh′	CDe/cDE	CcDEe	CcDEe
+	O	O	+	+	R^0r	R_0r	Rh_0hrhr	cDe/cde	ccDee	cDe
O	O	O	+	+	rr	rr hr″	hr′	cde/cde	ccee	ce
O	+	O	+	+	rr	rr hr′ hr″	rh′	Cde/cde	Ccee	Cce
O	O	+	+	+	rr	rr hr′ hr″	rh″	cdE/cde	ccEE	cEe
O	+	+	+	+	rr	rr rh″ hr′ hr″	rh′	Cde/cdE	CcEe	CcEe

between the D antigen sites probably accounts in part for the lack of complement fixation in D antigen–antibody reactions.

Weak D, D variant, Du, and partial D

Many red cell antigens show different degrees of reactivity, but this is more of a problem with the D antigen because all donors and patients are typed for D and it is important to choose D-negative red cells for D-negative patients. There are many phenotypes of D, and in some of these a portion of the D molecule may be missing. Some individuals who inherit the D antigen have red cells that react weakly or not at all with certain anti-D reagents. Thus, these individuals may appear to be Rh negative. This situation has been called D variant or Du, but the contemporary term is weak D. The weak D or partial D phenomenon can be caused by single

nucleotide mutations that change amino acids resulting in different insertions of the D protein in the red cell membrane or difference in exposure the protein or the number of available D antigen sites. Portions of the *RHD* gene can be replaced with portions of *RHCE* resulting in a hybrid D protein with fewer D antigen sites also called partial D. Very low levels of D (D-el) can be found in some apparently D negative individuals due to *RHD* mutations that reduce D expression. Alternatively, the presence of the Ce antigens in the trans position can reduce the expression of D. Thus, there are several different mechanisms leading to weak D expression [3]. These three forms of weak D occur in about 1% of all Rh-positive individuals. The gene interaction and weak D are more common than the D mosaic.

These observations are of intellectual interest and also have clinical impact. Persons who are weak D have the D antigen, although some may lack a part of it. Thus, the rare recipient who is weak D because of a partial absence of D and receives D-positive red cells could make anti-D. Recipients who are weak D because of D–Ce interaction have all the D antigens and can receive D-positive blood. However, it is not necessary to determine whether recipients who appear to be Rh negative are in fact weak D, because they will receive Rh-negative red cells since they type as Rh (D) negative. Weak D donors are of greater concern. They may type as Rh negative but contain some D antigen that could immunize an Rh-negative recipient. Thus, donor red cells must be tested for Rh (D) using methods that will detect the weak D antigens (see Chapter 7).

Rh null type

Rare individuals lack all the Rh antigens and the Rh protein. This may be due to the absence of the RHAG gene that regulates the expression of Rh antigens or to the presence of an amorphic gene at the RHD locus on chromosome 1 [3]. Rh-null individuals have abnormal red cell morphology and hemolytic anemia that is usually sufficiently compensated to result in only mild anemia. If exposed to Rh-positive red cells, these persons make an antibody that reacts with all red cells other than Rh null and is considered to be reactive against the entire Rh molecule.

Rh antibodies

The most common Rh antibody is anti-D. This is almost always an IgG, is reactive at 37°C, and does not bind complement effectively. The antibody causes HDN or serious transfusion reactions and red cell destruction by accelerated clearance of cells through the mononuclear phagocyte system (Table 9.6 and Chapters 12 and 14).

9.5 Other red cell blood groups

Kell system

The Kell blood group system was the first system to be identified using the antiglobulin test. Currently it includes 31 alloantigens, which includes five

Table 9.6 General laboratory reactions of some blood group antibodies.

RBC antigen	Protein type	Optimum temperature	Reaction medium	Binds complement	Causes HDN
ABO	IgM	20°C	Saline	Yes	Yes
Lewis	IgM	20–37° C	Variable	Yes	No
I	IgM	4°C	Saline	Some	No
P	IgM	4–20°C	Saline	Only anti-TJA	No
MN	IgM	4–20°C	Saline	Some anti-Ss	
Lutheran	IgM, IgA	Variable	Saline	Some or AHG	Occasionally
Rh	IgG	37°C	AHG	No	Yes
Kell	IgG	37°C	AHG	No	Yes
Duffy	IgG	37°C	AHG	No	Rare
Kidd	IgG	37°C	AHG	All	Yes

sets of antithetical antigens. The Kell antigens are numbered. The primary antigens of this system are Kell (K or KEL1) and its antithetical antigen Cellano (k or KEL2). Other antigens of the Kell system are Kp (a, b, and c), and Js (a and b). The antithetical sets are Kk, Kp(a)(b), and Js(a)(b). The K gene is an autosomal dominant whose frequency differs considerably in different populations.

The Kell gene is located on chromosome 7, has 2500 base pairs, and encodes for a 732-amino acid protein [26]. Polymorphism in the Kell system is due to single amino acid substitutions [3]. The Kell molecule is a 93-kD glycoprotein [26–28] that spans the membrane and has a large extracellular portion. The antigen reactivity probably depends on maintenance of the tertiary structure of the surface of the molecule. Kell is one of the first red cell antigens to appear on erythroid progenitors. Thus, in HDN due to anti-K, there may be less hemolysis but more suppression of erythropoiesis compared to anti-D [13].

The Kell system is important clinically because the K antigen is next in immunogenicity after D and because antibodies to Kell system antigens (or anti-K) are usually IgG, react at 37°C, and do not bind complement, but can cause severe hemolytic transfusion reactions and HDN (Table 9.6). It is not customary to type donors or recipients for Kell unless the recipient has a Kell antibody. The KK phentoype (k-) is very rare but anti-k, when it occurs, causes hemolytic transfusion reactions and HDN. A search for KK donor red cells can be difficult. Transient weak expression of Kell antigens occurs sometimes associated with bacterial infections. Often autoantibodies with Kell specificity are found in these patients [3].

The Kell glycoprotein is located very close to the integral membrane Xk protein that contains the Xk blood group antigen. Chronic granulomatous disease (CGD) is a congenital absence of a superoxide-producing cytochrome that interferes with the normal oxidative response of neutrophils so that their bactericidal activity is diminished and patients suffer severe, life-threatening infections. The genetic defect associated with CGD is on the X chromosome near the Kell-related locus, *Kx*. Rare

individuals who lack *Kx* are said to have the McLeod phenotype [29]. In the McLeod phenotype, the red cells express Kell system antigens weakly and have bizarre shapes, and patients have a chronic compensated hemolytic anemia. The occurrence of the McLeod phenotype in some patients with CGD led to the discovery that the McLeod syndrome involves a spectrum of abnormalities in addition to those involving the Kell system. Other associated abnormalities occur in red cells (acanthocytosis, compensated hemolysis, increased red cell phosphorylation) the nervous system (areflexia, choreiform movements, mild dysarthria, neurogenic myopathy, and muscle wasting), the cardiovascular system (cardiomyopathy), and granulocytes (CGD). Other changes such as reduced haptoglobin, elevated creatinine phosphokinase, lactic dehydrogenase, and carbonic anhydrase may be found.

Duffy system

Duffy was the first blood group locus to be identified on an autosome—chromosome 1. The system was first identified by finding anti-Fy^a in the serum of a transfused hemophiliac. The Duffy system is composed of six antigens: Fy^a, Fy^b, Fy^3, Fy^4, Fy^5, and Fy^6. The Duffy antigen molecule is a glycoprotein with a molecular weight of about a 36,000 kD. The molecule containing the Duffy antigens is called the Duffy antigen receptor for chemokines (DARC) (see section on Function). There are approximately 13,000 Fy^a or Fy^b antigen sites on each red cell in persons homozygous for the *Fya* or *Fyb* gene [30, 31]. Red cells from individuals heterozygous for *Fy^a* or *Fy^b* contain about 6000 antigen sites per red cell. These red cells often show a weaker agglutination than homozygous cells in serologic tests—a phenomenon called dosage effect. This can have a practical clinical effect. A patient with a weak anti-Fy^a might have a compatible crossmatch when serum is tested against Fy(a+b+) donor red cells. If an effective antibody screening procedure (see Chapter 7) was not carried out, the patient could receive Fya+ red cells that appeared to be compatible.

The Duffy system is unusual because the antigen frequency varies substantially in different racial groups. In general, the *Fya* gene has a high incidence in Asians and moderate incidence in Caucasians. A third allele *Fy* does not produce a Duffy molecule and such individuals are Fy(a−b−). This gene has a high incidence in Africans. Both Fya and Fyb antigens are detectable during fetal development and present in normal strength at birth.

Fy^a antibodies are rather frequently encountered in immunohematology laboratories and may cause hemolytic transfusion reactions or HDN. Although they may be severe, transfusion reactions to Fy^a are not frequent and the HDN is usually mild (Table 9.6). Anti-Fy^b is an infrequent cause of transfusion reactions and HDN. Other Duffy system antibodies have not been implicated in clinical problems.

One of the most interesting features of the Duffy system is its association with malaria. Fya(a−b−) red cells are resistant to infection by *Plasmodium knowlesi* [32] and *Plasmodium vivax*. The malaria parasites are unable to

penetrate red cells lacking the Fy^a and Fy^b antigens because they do not establish a junction site [33]. In West Africa most blacks have the Fy gene and are Fy(a−b−) and resistant to *P. vivax* malaria; thus, a natural selection process might have been in effect.

Kidd system

The three antigens of the Kidd system (Jk^a, Jk^b, and Jk^3) were discovered during the 1950s; no new antigens in this system have been reported since then. There is a rare null phenotype, Jk(a−b−), and Jk^3 is a high-incidence antigen. The Kidd glycoprotein is on the red cell urea transporter and is also found on neutrophils and renal medullary cells [34].

Red cells from individuals homozygous for *Jk*^{a} contain about 11,000 antigen sites [31]; Kidd antigens are well developed at birth. Kidd antigens may be altered by products of bacteria in infected patients. For instance, Proteus mirabilis and Streptococcus faecalis can cause Jk(b+) cells to become reactive with anti-Jk^b, and autohemolysis can occur.

Kidd antibodies are usually IgG but can also be a mixture of IgG and IgM. They often bind complement and can cause severe hemolytic transfusion reactions but usually cause only mild HDN (Table 9.6). In vitro Kidd antibodies may react weakly and yet cause severe hemolysis despite being IgG. The relatively low number of Kidd antigen sites [11,000] should mean that the distance between antigen sites is too great for complement activation by an IgG molecule; however, the antigens probably are clustered, thus accounting for weak serologic reactivity, complement activation by IgG, and severe clinical effects.

A unique characteristic of Kidd antibodies is that they often disappear. Thus, they may cause delayed hemolytic transfusion reactions (see Chapter 14). This occurs when the patient has been immunized previously by pregnancy or transfusion but no longer has circulating antibody, so all the pretransfusion tests are compatible; yet if the patient receives Kidd-positive red cells, the antibody may be resynthesized rapidly, causing hemolysis of the transfused red cells a few hours to days later. Another feature of Kidd blood group serology is the phenomenon of "dosage." That is, anti-Jk^a may react more strongly with red cells homozygous for Jk^a than heterozygous Jk(a+b+) cells. Thus, the antibody can be missed in laboratory tests if homozygous antibody detection cells are not used (see Chapter 8).

Lutheran system

The Lutheran system has 19 antigens including four anti-thetical pairs and others of high prevalence. The four pairs are Lua/Lub; Lu6/Lu9, Lu8/Lu14, and are a Aub. The Lutheran system was known to be linked to the third component of complement, and the location of the C3 gene to chromosome 19 led to the localization of the Lutheran gene to that same chromosome [35]. Lutheran antigens are part of a glycoprotein molecule of 78–85 kD that traverses the red cell membrane. The number of antigen sites has been reported to be 1000–4000 per cell, depending on the zygosity. The Lutheran glycoprotein is part of the immunoglobulin

superfamily of molecules that function as receptors or adhesion molecules. Lutheran antigen strength (number of sites) varies among families and among red cells of an individual. Thus, Lutheran typing may show a mixed field pattern of reactivity.

The antigens are not well developed at birth, and so antigens and antibodies of the Lutheran system cause only mild hemolytic disease of the newborn. Lutheran antibodies may be IgG or IgM or mixtures of these with IgA (Table 9.6). The most common Lutheran antibodies are anti-Lu^a and anti-Lu^b. Anti-Lu^a is usually not active at body temperatures and thus is not a problem clinically. These antibodies have not been implicated in immediate hemolytic transfusion reactions or in HDN requiring exchange transfusion. Anti-Lu^a is rarely found because the incidence of Lu(a+) red cells is low; thus, although most patients are Lu(a−), they are rarely exposed to the antigen. Laboratory test cells often are not Lu(a+), so the antibody is not detected. Fortunately, even if the antibody is missed in the antibody detection test, patients with the antibody would rarely receive a transfusion of Lu(a+) red cells. Anti-Lu^b may cause accelerated destruction of incompatible red cells. However, this antibody is also rarely found because almost all individuals are Lu(b+).

MNSs system

The M and N blood group was the second system discovered. This was done by immunizing rabbits with human red cells. The system has five major antigens: M and N, which are antithetical; S and s, which are antithetical; and U, which is present in all individuals who possess M or N and S or s antigens and in some S-s- individuals. They are considered part of the same blood group system because they result from closely linked loci [3]. In addition to these five antigens, this system has many variants and includes 46 different antigens. The composition of antigens of this system is well understood. The antigens of the MNSs system are located on the red cell membrane terminal portion of the sialic acid-rich glycoprotein called glycophorin A, of which there are from 200,000 to 1,000,000 copies per red cell. Glycophorin A has a molecular weight of 43,000 and is composed of 131 amino acids. The MN activity is located in approximately 70 external amino acids, the remaining amino acids being intramembranous or intracytoplasmic. The Ss and probably the U antigens are located on the terminal portion of glycophorin B, of which there are 50,000–250,000 copies per red cell.

Antibodies to the MN antigens are usually not clinically active. Although anti-M is often IgG, most MN antibodies have a large IgM component, are usually not active at body temperatures, and only rarely cause hemolysis, transfusion reactions, or HDN (Table 9.6). Anti-M may be found in persons who have not been previously transfused. Unusual examples of anti-M or N may be active at 37°C and clinically significant. Anti-N is almost always IgM, not reactive at body temperature, and not clinically significant. Anti-S, anti-s, and anti-U are usually IgG warm active antibodies that may have clinical effects by causing transfusion reactions,

hemolysis, or HDN. Anti-S occurs infrequently, anti-s is rare, and anti-U is extremely rare.

P system

Like the MN system, P-system antigens were discovered before the Rh system by injecting animals with human red cells. The P system contains only the P_1 antigen. Other antigens, P, Pk, and LKE, have serologic relationships to P_1 but are not controlled by genes at the same locus as P_1 and are called a "collection" [1–3]. All but 1 in 100,000 persons have the P antigen. The two most common phenotypes are P1 (P1P) and P2 (PP); each of these phenotypes has trace amounts of Pk. The amount of P1 antigen varies greatly among individuals, is not well developed at birth, and does not reach adult levels until the individual is about 7 years of age. P-negative (pp) individuals lack P1, P, and Pk and are very rare.

The P-system antigenic determinants are carbohydrates that are linked to glycosphingolipids in the red cell membrane. P-system antigens, like the ABO and I antigens, seem to be formed by the sequential addition of monosaccharides to a precursor instead of being antithetical and controlled by allelic genes. The biochemistry of P-system antigens is better understood than the genetics. Genetic control of the P system has been proposed to involve two or three genetic loci that code for enzymes that add the carbohydrates to the lipids.

Antigen structures similar to those of the P system are found in animal tissue, pigeons, doves, worms, and Echinococcus cyst fluid. Thus, many antibodies of the P system are called "naturally occurring," although as with ABO antibodies this is not technically correct. Because some bacteria, viruses, or parasites have antigen structures similar to P-system antigens, the P system may play a role in certain diseases (see section on Function), and anti-P1 is sometimes found in patients with parasitic infections.

Antibodies of the P system are rather common and can range from cold active and clinically insignificant to those that cause severe hemolysis (Table 9.6). Anti-P1 is common, usually cold active, and not clinically significant unless it reacts at body temperatures. Anti-P1+P+Pk made by people with the rare pp phenotype and anti-P made by people with the rare pk phenotype will cause hemolysis in vitro and in vivo. There is a high incidence of miscarriage in women with the pp phenotype, and it has been suggested that anti-P+P1+Pk may have a pathologic role. Auto anti-P is the specificity of the Donath–Landsteiner antibody found in paroxysmal cold hemoglobinuria. The antibody is often called biphasic because it binds to the red cell at cool temperatures, then causes hemolysis at warmer (body) temperature.

Lewis system

The gene for the Lewis system is linked to the third component of complement and is located on chromosome 19. The Lewis system is not a blood group system. Lewis antigens are probably synthesized in the intestinal epithelium, are soluble in body fluids, and the antigens present in plasma are adsorbed onto red cells. There are two antigens in the

Lewis system: Le^a and Le^b. The Lewis antigenic specificity is determined by the carbohydrate fucose. The Le gene produces a glycosyltransferase that adds the fucose to a precursor substance [type 1 chain] to produce the Lewis antigen, which is a glycosphingolipid. The Lewis system also differs from most other blood group systems in that the Lewis genes must interact with Hh and secretor genes in order for Lewis antigens to be produced. However, the exact Lewis antigen produced is determined by the presence or absence of the secretor gene (Se). Se determines the location on the precursor substance (type 1 chain) to which the fucose is added by the Le gene's enzyme. If both the Lewis and Se genes are present, both Le^a and Le^b are added and the individual is Le(a+b+). However, if only the Lewis gene is present (the individual is sese and lacks the Se gene), Le^b is not produced and the individual is Le(a+b−). Thus, two different antigen specificities are produced depending on whether the secretor gene is present. In addition, the ABH makeup of the individual determines the position and linkage of the fucose being added by the Lewis gene, and this creates several variations of the Lewis phenotype. Because of the involvement of the secretor gene, Lewis substance is also released into the plasma and saliva, which are of primary practical interest in transfusion medicine, but also into milk, gastrointestinal fluids, urine, seminal fluid, ovarian cyst fluid, and amniotic fluid [3].

Newborns' red cells lack Lewis antigens and there is no Lewis substance in their plasma, although Lewis substance is present in their saliva. The adult Le^a antigen phenotype is attained by about 1 year of age but not until about 7 years for Le^b. Lewis antigens are decreased during pregnancy because there is an increased lipoprotein to red cell mass, which results in a shift of glycolipids from red cells to plasma, reducing the Lewis antigen content on the red cells. Also, the Lewis type of transfused red cells may change. After transfusion, the transfused donor red cells convert to the Lewis type of the recipient. Thus, Lewis antigen may be acquired or lost through the exchange of glycolipids between the plasma and red cell.

Lewis antibodies are common, usually react below body temperature, are mainly or entirely IgM, and are not clinically significant (Table 9.6). These antibodies are usually found in patients who have not been transfused and are thus sometimes called "natural"—a technically incorrect term, since they are probably stimulated by environmental agents. Anti-Le^a is often found in pregnant women, but it is not clear that the antibody is formed after immunization by fetal red cells. Lewis antibodies do not cause HDN because they are usually IgM and also Lewis antigens are not present on fetal red cells. A few examples of anti-Le^a have caused hemolytic transfusion reactions. In these situations, usually the antibody reacts at body temperatures, and this can be used as an indication of clinical significance. Other reasons that Lewis antibodies usually do not cause hemolysis are that the patient's antibodies are partially neutralized by the small amount of soluble Lewis substance in the plasma of the donor, and the Lewis antigens on the transfused red cells elute into the plasma of the recipient. Lewis antibodies have been implicated in the rejection of transplanted Lewis-positive kidneys [36], but Lewis matching of donated kidneys is not done in most transplant programs.

LW system

Although LW is a genetically distinct system, this was not recognized for years. Because the expression of LW and Rh is related, LW was thought to be part of the Rh system. LW antigen is named for Landsteiner and Wiener because it was the antigen defined on red cells by the original antibody prepared in rhesus monkeys [18]. Adult D-positive red cells exhibit stronger LW activity than D-negative red cells. Thus, weak examples of anti-LW appear similar to anti-D because they react with D-positive but not with D-negative red cells. This was the situation with the original anti-LW serum, and therefore it reacted similarly to the serum found by Levine and Stetson in their original report of HDN [17]. Thus, the name of the Rh system was from the LW antibody produced in rhesus monkeys but that was in fact an antibody to a different antigen in a different system (LW). The original serum reacted with what is now known as the LW^a antigen. LW^b has been identified and four phenotypes have been observed: LW(a+b−), LW(a+b+), LW(a−b+), and LW(a−b−). The molecule is a 42 kD glycoprotein and is a member of the intracellular adhesion molecule (ICAM).

LW antibodies are very rare. Only very rare individuals lack LW^a antigen and can make anti-LW^a. LW antibodies are usually not clinically significant but may rarely cause accelerated destruction of LW-positive red cells [3]. Anti-LW may also occur as a transient autoantibody in persons who temporarily lose LW antigen activity. This loss may occur during pregnancy or in association with malignancy [3].

Diego (Di)

The Diego system includes two pairs of antithetical antigens: Di^a and Di^b and (Wright) Wr^a and Wr^b plus 17 other low-frequency antigens. Di^a was the first of these to be discovered and is common in South American Indians but rare in European Caucasians. The Diego antigens are part of band 3 of the red cell membrane. Anti-Di^a is not a common antibody because of the rarity of the antigen in the United States; however, the antibody is clinically significant and can cause HDN but usually not red cell destruction. Anti-Wr^a is common but is often not detected because most red cells used for antibody detection lack the antigen. Although anti-Wr^a can cause HDN and transfusion reactions, because of its rarity, special efforts are not made to include Wr^a positive cells on antibody screening panels.

Cartwright (Yt)

The Cartwright system consists of two antigens, one of high incidence and one of low incidence. The antigens are located on red cell acetylcholinesterase. Many examples of anti-Yt^a have been reported but anti-Yt^b is very rare. Anti-Yt^a has not caused HDN and usually does not cause hemolysis or transfusion reactions, but occasional examples do cause accelerated red cell destruction [3].

Xg system

An antibody that reacted more frequently with red cells from females than those from males identified the antigen named Xg^a. The Xg^a gene is carried on the X chromosome, and about 65% of males and 89% of females are Xg^a positive. The antithetical antigen has not been found. Anti-Xg^a does not cause HDN or hemolysis and thus is not considered to be clinically significant.

Dombrock (Do)

The Dombrock system includes the Do^a and Do^b and three other high-frequency antigens. The antigen is located on a glycoprotein of the ADP-ribosyltransferase family. Dombrock antibodies are not common, although anti-Do^a and Do^b have caused mild HDN or red cell destruction [3].

Scianna (Sc)

The Scianna system has seven antigens; Sc1 is a high-incidence antigen, and the antithetical antigen of low incidence is Sc2. An additional antigen, Sc3, is present on the cells of anyone who inherits either Sc1 or Sc2. The low-incidence antigen Radin also belongs to the Sc system. Sc antibodies are rare and have not been reported to cause HDN or accelerated red cell destruction.

Colton (Co)

The Colton system includes a high-incidence antigen (Co^a) and a low-incidence antigen (Co^b). The system also resembles Scianna and Duffy, in that there is a third antigen (Co3) that is present whenever there is a functional Co gene. Colton antigens are part of the water transport protein Aquaporin 1, also known as the channel-forming integral protein (CHIP). Colton antibodies are rare, but anti-Co^a has been implicated in HDN and red cell destruction.

Rogers (Rg) and chido (Ch)

The fourth component of complement (C4) is polymorphic. The Ch and Rg antigens are present on the C4A plasma components that remain attached to the red cell membrane. Thus, these antigens are not integral to the red cell membrane. There are nine antigens in the Ch/Rg group. Therefore, individuals who lack all Ch and Rg antigens also lack C4 and have a high incidence of systemic lupus erythematosus. Antibodies to Ch and Rg antigens are not clinically significant because they have not been involved in HDN or red cell destruction. They can create problems in laboratory testing (see Chapter 10).

Gerbich (Ge)

The Gerbich system contains three antigens of high incidence and five of low incidence. The antigens are located on glycophorin molecules. Gerbich system antibodies are of variable clinical significance. Some have caused mild HDN or slightly accelerated red cell clearance. Very rarely, the

autoantibody in patients with autoimmune hemolytic anemia may have Gerbich specificity [3].

Cromer (Cr)

Fifteen different antigens have been assigned to the Cromer system. The antigens are located on the decay-accelerating factor (DAF), which is a complement regulatory protein that is attached to the red cell membrane by a glycosylphosphatidylinosotal linkage. The antibodies have varying clinical significance. None has caused a transfusion reaction or been implicated in HDN, although some Cromer antibodies have caused accelerated red cell destruction [3].

Knops (Kn)

There are nine antigens of the Knops system. These include the Helgeson and McCoy phenotypes. The Knops antigens are located on the CR1 complement receptor, which is the primary complement receptor on red cells. Knops system antigens have different strengths of reactivity, and this is based more on the amount of red cell CR1 than on antigen dosage. Antibodies of the Kn system, like those of Chido and Rogers, have serologic reactivity referred to in the past as "high titer, low avidity." The sera caused weak reactivity, but the reactivity remained even after the serum was extensively diluted. Clinically, the antibodies are not significant because they do not cause transfusion reactions, increased red cell destruction, or HDN. However, they may be clinically significant in the broad sense that they make it difficult to carry out serologic investigations and their reactivity may mask other clinically dangerous antibodies. The CR1 receptor is involved with *Plasmodium folciparum* attachment to red cells.

Indian (In)

There are four antigens of the Indian system: one of low incidence (In^a) and one of high incidence (In^b). The antigens were discovered in people from the Indian subcontinent, and thus the source name. The antigens are located on a glycoprotein that is a cellular adhesion molecule and lymphocyte homing receptor. A high-frequency antigen AnWj is not part of the Indian group but is located on the same glycoprotein. The In glycoprotein is present on many tissues, including red cells. Neither anti-In^a nor anti-In^b has been implicated in HDN, but both have caused a transfusion reaction and decreased red cell survival. Thus, they should be considered clinically significant.

OK system

There is only one antigen (OK^a) in this system, which is located on the immunoglobulin superfamily molecule [3]. OK^a negative red cells have been found only in eight Japanese families. The antibody caused a shortened red cell intravascular survival but did not seem to be clinically significant in these families [3].

RAPH system

Although this system is named after the individual with the first antibody, the only antigen in this system is called MER2 [3]. The antigen was the first to be identified using monoclonal antibodies. Anti-MER2 is not clinically significant.

JMH system

This system now has five antigens—John Milton Hagen (JMH) was the original antibody and the JMH negative state is inherited in only one family. Most of the other JMH negative individuals are elderly and all seem to have lost the JMH antigen to acquire the negative state. The antibody is not clinically significant, although red cell survival has been shortened in some studies [3].

GIL

The GIL antigen is located on the aquaporin-3 molecule, which is a channel for red cell membrane permeability. GIL antibodies are reactive in the antiglobulin test and detect the very high frequency antigen. There have not been any reports of hemolytic transfusion reactions or HDN due to anti-GIL.

Ii blood group antigens

The I and i antigens are part of the interior of the oligosaccharide chain that contains the ABH and Lewis antigens. One of the unique things about this system is that the presence of the I and i antigens changes with age. At birth, the oligosaccharides have the i structure and the action of the *I* gene causes branching of the linear i oligosaccharide chain to create the I phenotype. In most adults, very little i is detectable, except for rare individuals who remain i positive and I negative. I specificity is conferred by the addition of a D-galactose-*N*-acetyl-D-galactosamine to the i oligosaccharide chain. Thus, it appears that I and i are not alleles but are part of a sequence of steps. The antigen structure is a complex branched chain, and it has been suggested [3,4] that the variety of antibodies that occurs in the I system is due to the formation of antibodies against domains within the antigen molecule. Other I system antigens are rare forms, transitional forms, or a complex with other antigens. The amount of Ii antigen on red cells varies in different individuals, and this also leads to the appearance of different forms of I antigens. I and i antigens are present in a soluble form in serum, saliva, breast milk, urine, amniotic fluid, ovarian cyst fluid, and hydatid cyst fluid. Patients with dyserythropoietic conditions or undergoing repeat phlebotomy may have elevated expression of i.

   The major antibodies of this group are anti-I and anti-i. These are usually autoantibodies that are optimally reactive at cold temperatures and not clinically significant. They do not cause HDN, and there is only one report of an anti-I that caused accelerated destruction of I red cells [37]. Almost all normal adults' serum contains anti-I; however, it is also found in cold agglutinin disease and transiently in Mycoplasma pneumoniae infection. In cold agglutinin disease, the antibody changes characteristics

and is an auto-anti-I, which reacts at body temperature, fixes complement, and causes hemolysis. Anti-i is rare but has been associated with infectious mononucleosis.

HLA

The HLA system is described in more detail in Chapter 16.

9.6 Antibodies to red cell antigens

Antibodies to red blood cell antigens vary widely in their characteristics and in vivo significance [38]. Red blood cell antibodies can be categorized based on their immunoglobulin class, the antigen to which they are directed, their optimum temperature of reaction in vitro, whether they fix complement, their action on red cells in vitro, and their in vivo effect. A summary of the general characteristics of antibodies of the major red blood cell antigen systems is provided in Table 9.6; however, specific antibodies within each system may exhibit different characteristics, so an appropriate reference text should be consulted before decisions are made regarding the identity or potential clinical effect of a particular antibody.

Almost all red cell antibodies are either IgG or IgM. There are rare examples of IgA red cell antibodies. There are some clinical and laboratory differences between IgM and IgG red cell antibodies (Tables 9.6 and 9.7). The mechanism of action of these antibodies is that the Fab portion of the Ig molecule binds to the antigen-combining site on the red cell surface. The avidity and binding constants for this antibody–antigen reaction vary widely for different red cell antibodies. After binding, the heavy chain region of the antibody molecule determines the biologic effect of the antibody by activating complement or reacting with receptors in the fixed macrophages of the liver and spleen to cause accelerated red cell clearance.

The specificities of the most common red cell antibodies found in hospitalized patients, pregnant women, and normal blood donors differ slightly because of the nature of these populations. The incidence of different antibodies depends on the prevalence of the antigen in the population and the immunogenicity of the antigen. Factors that determine

Table 9.7 Methods of characterizing red cell antibodies.

Physiochemical	IgG, IgM, IgA
Antigen	Iso, auto, hetero
In vitro action	Agglutinating (complete)
	Coating (incomplete, blocking)
	Complement fixing (hemolysin)
Temperature of optimum reaction	Warm, 37°C
	Cold, 20–4°C
Optimum method of detection	Saline medium
	High-protein medium
	Antiglobulin serum required

immunogenicity are not fully understood. The clinical importance of an antibody depends on whether it is likely to cause red cell destruction and also on the incidence of the antigen. For instance, the combination of the 15% incidence of Rh-negative individuals, the 65% likelihood of becoming immunized after exposure to one unit of Rh-positive blood, and the serious clinical effect of anti-D makes the Rh(D) antigen the single most important non-ABO antigen.

9.7 Function of molecules containing red cell antigens (see reference 13 for summary)

The clinical effects of most red cell antigens arise because they serve as targets for antibodies, and thus the effects are due to their corresponding antibodies. These effects have been described above with each blood group system. The laboratory detection of these blood group antigens and antibodies has been incorporated into a working system to provide safe and effective red cell transfusions.

Red cell blood group antigens have also been of interest to geneticists because of their ability to serve as a genetic marker. The chromosomal location of all blood groups has been established (Table 9.2), and linkages between some blood groups and diseases are now known. Studies of the red cell membrane have also yielded information about the molecules that contain the red cell (blood group) antigens and they are recognized increasingly as parts of important structural or functional components of the cell membrane. Thus, it is not surprising that certain rare red cell antigens or alterations in red cell antigens may be associated with altered membrane constituents, red cell morphology, or diseases (Table 9.8).

Red cell structure

Many red cell antigens are located on molecules that are essential for normal red cell structure. Alterations of these molecules may cause membrane abnormalities leading to shortened red cell survival (hemolytic anemia) or unusual morphology. Some clinical conditions such as malignancies, stress, and pregnancy may alter the expression of blood groups (Table 9.9). Glycophorins are major structural proteins on the red cell surface, accounting for most of the negative surface charge that may function to prevent undesirable cell-to-cell interactions. The MN antigens are part of glycophorin A, the Ss antigens are part of glycophorin B, and the Gerbich antigens are part of glycophorins C and D [39]. Rare variants of these blood group antigens are helpful in elucidating the role of glycophorins in red cell structure and function. Red cells that lack glycophorin A (the En(a−) phenotype) or glycophorins A and B (the MkMk phenotype) are apparently normal. However, those that lack glycophorins C and D (Leach phenotype) lead to elliptocytes and decreased deformability. These glycophorins may also be part of a membrane complex that includes band 3, the glucose transporter, and the Rh, Kell, Duffy, and, Xr molecules [13]. The Diego antigen also has a

Table 9.8 Rare blood types associated with abnormal morphology or disease.

Antigen or phenotype	Biochemical modification	Abnormality
Rh_{null}	No Rh or LW antigens	Stomatocytes, spherocytes, partially compensated hemolytic anemia
McLeod	Weak expression of normal	Acanthocytes; partially compensated hemolytic anemia
	Kell antigens; Kx absent	
K_o	No normal Kell antigens; Kx present	None observed
P	No normal P antigens; decreased globoside and ceramide trihexoside	None observed
Pk	Normal P1 antigen; CTH increased; globoside decreased	None observed
$P1^{pk}$	Increased GL3 (NAc galactose transferase deficiency)	None observed
Lu(a−b−)	Not studied	Abnormal scanning electron microscopy: wrinkled, furrowed appearance
En(a−)	Glycophorin A absent; depressed MN antigens; enhanced Rh(D) antigen	None observed
S-s-	Glycophorin B absent or abnormal	None observed
M^kM^k	Both glycophorin A and glycophorin B absent	None observed

Source: Adapted with permission from McCullough J. Transfusion Medicine. In: Handin RI, Lux SE, Stossel TP, eds. Blood: Principles and Practice of Hematology. Philadelphia: Lippincott, Williams, and Wilkins, 2003:2028.

structural function [13]. The Lutheran and X4 proteins interact with spectrin and red cells with reduced Lutheran have abnormal morphology. The RAPH antigens are on a molecule that is important in the integrity of basement membranes. The role of band 3 and the Gerbich blood group has already been mentioned.

Red cell function

Many red cell blood groups are part of molecules that have important red cell functions. These functional molecules involve serving as receptors, transport proteins, complement proteins, adhesion molecules, enzymes, or microbial receptors (Table 9.10). Usually the red cell antigen is not directly involved in the function but happens to be located on the same molecule.

Receptors and adhesion molecules

Red cells bind chemokines such as interleukins, and it has been noted that Duffy (a−b−) red cells failed to bind the chemokine IL-8. Thus, the Duffy blood group serves as a chemokine receptor [40]. It appears that the role of the red cell chemokine receptors is to bind and thus inactivate chemokines in the blood. The Indian blood group antigens are located on the adhesion molecule CD44 [41]. CD44 is involved in the adhesion of lymphocytes to endothelium and thus is involved in lymphocyte homing to lymphoid tissues. Also of interest is the possibility that CD44 may be involved in the

Table 9.9 Clinical conditions with red cell antigen alteration.

| Clinical condition | Red cell antigen | | Red cell antibody |
	Depressed	Increased	
Autoimmune hemolytic anemia	Rh-LW	I	Autoanti-D, LW
			Anti-Kp[b]
Leukemia with monosomy 7	ABH	IiH	
	Co[a]		
Myelofibrosis	LW		
	Rh		
	ABH		
Hodgkin's disease			Anti-LW
Paroxysmal nocturnal hemoglobinuria		i	
Paroxysmal cold hemoglobinuria			Autoanti-P
Ovalocytosis	Rh, LW	H	
	Ss, U		
	Kidd		
	Yt[a], Xg[a]		
Chronic granulomatous disease	Kell		Anti-KL + Kx
Hereditary hemolytic anemia	Rh		
	Kell		
	Colton		
Sickle cell anemia—stress		i	
Pregnancy	LW		Anti-LW
	Lewis		Anti-Lewis
	Sd[a]		
Bacterial infections			Acquired B antigen
			T activation
Chromosomal defects	Rh		
	MN		
	Duffy		

Source: Adapted with permission from McCullough J. Transfusion Medicine. In: Handin RI, Lux SE, Stossel TP, eds. Blood: Principles and Practice of Hematology. Philadelphia: Lippincott, Williams, and Wilkins, 2003:2028.

homing of hematopoietic progenitor cells to marrow extracellular matrix. The LW blood group antigen is located on a membrane protein with a structure similar to ICAM 2, which is part of a group of molecules that bind leukocytes to endothelium. It is not known whether the LW antigen is actually involved in adhesion. The Lutheran antigen is also part of a membrane-spanning protein and may be active in adhesion, but this has not been established. The Lutheran, LW, and OK glycoproteins are in molecules that are part of the immunoglobular superfamily, which is a large family of receptors and adhesion molecules whose primary function is not known.

Transport protein

Specific (transport) molecules are responsible for transporting nutrients into the cell and waste products out of the cell. Several blood groups are

Table 9.10 Functions of molecules containing red cell blood group antigens.

Function	Blood group
Receptor	Duffy, Knops, Indian, LW, Lutheran, OK
Transport protein	Diego, Wright, Colton, Kidd, Gil
Complement pathway	Chido, Rogers, Cromer, Knops
Adhesion molecule	Indian, LW, Lutheran
Structural integrity	MN, Ss, Gerbich, Kell, Duffy, Lutheran, Xr, RAPH
Enzymatic activity	Cartwright, Kell, Dombrock?
Microbial receptor	Duffy, P, Cromer, Le(b), Knopps, AnWg

located on these molecules. One major transport molecule is band 3, a transmembrane protein that traverses the membrane multiple times, and is the major anion exchanger. The Diego and Wright blood groups are located on band 3. Because the Wright antigens require expression of glycophorin A, the transport function of band 3 is reduced when red cells are glycophorin deficient. The major water channel protein is the CHIP. The gene coding for this protein is located on chromosome 7 near the gene for the Colton blood group and Colton antigens are located on the CHIP molecule [42]. The Kidd antigens are probably part of a molecule that acts as a urea transporter, because Jk(a−b−) cells are resistant to urea lysis. Rh and Kx and Rh may also be involved with membrane transport because portions of the molecules on which they are located span membranes.

Complement regulatory molecules

Three blood group systems are part of molecules involved in the complement pathway. The Chido/Rogers antigens are part of the C4 molecule [43]. The C4 molecule and these antigens are absorbed onto red cells from the circulation. The Cromer antigens are located on the DAF red cell membrane molecule [44], and the Knops antigens are part of the C3b complement receptor [45]. The Cromer antigens were helpful in elucidating the cause of paroxysmal nocturnal hemoglobinuria (PNH). The role of DAF is to protect red cells from complement damage, and it was hoped that the rare Cromer-negative individual would establish the key role of the absence of DAF as the cause of PNH. However, Cromer-negative individuals have only a mildly increased sensitivity of complement. This finding led to the discovery that another molecule (CD59) was the key in PNH.

Enzymatic activity

The Cartwright blood group antigens are located on the acetylcholinesterase molecule [46]. The role of this enzyme molecule on the surface of red cells is not known. The Kell glycoprotein is an endopeptidase that produces the vasoconstrictor, endothelin. The Dombrock glycoprotein may be an ADP-ribosyltransferase.

Microbial receptor

As a first step in infection, an invading organism must bind to tissues. Molecules are not only chemokine receptors but are also involved in malaria. Duffy (a−b−) red cells are resistant to infection by *P. vivax* because the parasites cannot establish a junction site on the red cell surface in the absence of the Duffy structure [30, 32, 47]. Escherichia coli have several adhesion molecules. One group of these binds to the DAF glycoprotein, which contains the Cromer blood group. It appears that the Cromer antigen is the receptor for this binding and thus facilitates infection with E. coli [48]. The DAF molecule containing Cromer is located on cells other than red cells, and there is a suggestion that binding of E. coli to Cromer in the urinary tract facilitates urinary tract infection. This mechanism is more clear with the P blood group system. Some E. coli adhesion molecules bind to the glycosphingolipids of the P system, thus increasing the likelihood of urinary tract infection [49]. There are associations between le(b) and Helic diacter pylori, Knopps, and Mycobacterium leprae, AnWy with hemophilis influenza, and antigens of the glycophorins A, B, C, D with Plasmodium falciparum [13]. Some viruses also adhere to molecules containing blood group antigens. The parvovirus B19 adheres to globoside of the P blood group system [50]. Enteroviruses such as coxsackievirus or echovirus use DAF containing the Cromer antigens as receptors. Poliovirus may use the CD44 molecule containing the Indian blood group antigens in its adhesive process.

9.8 Platelets

Platelets contain antigens from several red cell blood group systems, including ABH, Lewis, Ii, and P. Platelets do not contain Rh, Duffy, Kell, Kidd, and Lutheran antigens. It appears that some ABH antigens are intrinsic to the platelet membrane and some are adsorbed onto the cell surface from the plasma, which contains soluble A and B substance [51]. The density of A antigen sites on platelets is only about 5% of that on red cells [51]. ABO antigens have some importance in platelet transfusion in that it appears that ABO incompatibility reduces the response to platelet transfusion (see Chapter 11).

In contrast to ABH antigens, HLA antigens are integral to the platelet membrane. However, only HLA class I (A, B, and C locus) [52] and not class II (DR) antigens are present. The HLA system is discussed more extensively in Chapter 16, and the clinical impact of the HLA antigens on platelets is discussed in Chapter 12.

In addition to red cell and HLA system antigens, there are several antigen systems that are platelet specific because they are polymorphisms on platelet glycoproteins [53, 54] (Table 9.11). The antigens were discovered by studies of sera from mothers who delivered thrombocytopenic infants. The mother's sera reacted against platelets of the infant and the infant's father in a manner similar to red cell hemolytic disease of the newborn. Much like red cell antigens, the initial names given

Table 9.11 Human platelet alloantigens.

System	Antigen	Alternative names	Glycoprotein	Nucleotide change	Amino acid change	Molecular name	Phenotype prevalence
HPA-1	HPA-1a	Zw^a, PI^{A1}	GPIIIa	T^{196}	Leu^{33}	GPIIIa Leu33	97.9
	HPA-1b	Zw^b, PI^{A2}		C^{196}	Pro^{33}	GPIIIa Pro33	28.6
HPA-2	HPA-2a	Ko^b	$GPIb^a$	T^{524}	Thr^{145}	$GPIb^a$ Thr145	99.9
	HPA-2b	Ko^a, Sib^a		C^{524}	Met^{145}	$GPIb^a$ Met145	13.2
HPA-3	HPA-3a	Bak^a, Lek^a	GPIIb	T^{2622}	Ile^{843}	GPIIb Ile843	80.9
	HPA-3b	Bak^b		G^{2622}	Ser^{843}	GPIIb Ser843	69.8
HPA-4	HPA-4a	Yuk^b, Pen^a	GPIIIa	A^{526}	Arg^{143}	GPIIIa Arg143	99.9
	HPA-4b	Yuk^a, Pen^b		G^{526}	Gln^{143}	GPIIIa Gln143	0.0
HPA-5	HPA-5a	Br^b, Zav^b	GPIa	G^{1648}	Glu^{505}	GPIa Glu505	99.0
	HPA-5b	Br^a, Zav^a, Hc^a		A^{1648}	Lys^{505}	GPIa Lys505	19.7
HPA-6W	HPA-6bW	Ca^a, Tu^a	GPIIIa	C^{1564}	Arg^{489}	GPIIIa Arg489	0.7
				G^{1564}	Gln^{489}	GPIIIa Gln489	
HPA-7W	HPA-7bW	Mo^a	GPIIIa	G^{1317}	Pro^{407}	GPIIIa Pro407	Unknown
				C^{1317}	Ala^{407}	GPIIIa Ala407	
HPA-8W	HPA-8bW	Sr^a	GPIIIa	C^{2004}	Arg^{636}	GPIIIa Arg636	Unknown
				T^{2004}	Cys^{636}	GPIIIa Cys636	
HPA-9W	HPA-9bW	Max^a	GPIIb	A^{2306}	Val^{837}	GPIIb Val837	Unknown
				G^{2306}	Met^{837}	GPIIb Met837	
HPA-10W	—	La^a Lab^b	GPIIIa	—	Arg Gln^{62}	— —	<1 >99
—	—	Gro^a	GPIIIa	A^{1996}	Arg^{633}	Arg633 GPIIIa	Unknown
				G^{1996}	His^{633}	GPIIIa His633	
—	—	Va^a	GPIIIa	—	—	—	—
—	—	Ly^a	GPIb/IX	—	—	—	—
—	—	Pe^a	GPIba	—	—	—	—
—	—	Oe^a	GPIIIa	—	—	—	—

Sources: von dem Borne AEGKr, de Haas M, Simcek S, Porcelijn L, van der Schoot CE. Platelet and neutrophil alloantigens in clinical medicine. Vox Sang 1996;70(Suppl 3):34–40 and Technical Manual, American Association of Blood Banks, 14th ed. Bethesda, MD, 2003.

Table 9.12 Clinical situations involving platelet-specific antigens and antibodies.

Autoantibodies	Autoimmune thrombocytopenia
	Drug-induced immune thrombocytopenia
Alloantibodies	Neonatal alloimmune thrombocytopenia
	Posttransfusion purpura
	Refractoriness to platelet transfusion

to platelet-specific antigens often were related to the patients in whom the antibody was found. In 1990, a new nomenclature system was developed that used the prefix HPA for human platelet antigen and assigned a number to each locus followed by a letter to designate each antigen [55] and this is now updated [54]. Thus, HPA 1a is the first antigen at the first locus. This antigen corresponds to the original antigen PlA1 (Table 9.11). At present there are six pairs of alloantigens for a total of twelve antigens. These antigens are located on four different glycoproteins (Table 9.11). Other individual antigens that appear to be platelet specific have been classified into 11 additional systems with a single low frequency antigen for a total of 23 antigens (Table 9.11). The platelet polymorphisms are located on GPIIb/IIIa, GPIa/IIa, GPIb/IX/v, and CD19 glycoproteins.

These platelet-specific antigens have clinical importance (see Chapter 12 and Table 9.12). They are targets for autoantibodies, alloantibodies, and drug-dependent antibodies. These platelet-specific antibodies may cause autoimmune thrombocytopenia or several different clinical problems or diseases due to alloimmunization. Rather surprisingly, it appears that platelet-specific antigens and antibodies are not of major importance in alloimmunization to platelets and refractoriness to platelet transfusion or in hematopoietic stem cell transplantation [56] (see Chapter 11). Because platelet glycoproteins function as receptors in the hemostatic process, future studies may demonstrate that abnormalities of these molecules cause alterations in platelet antigens or that altered platelet antigen strength or specificity may affect the structure or function of the cell.

9.9 Granulocytes

Alloantigen systems of interest that might be found on granulocytes include red cell antigens, HLA antigens, and granulocyte-specific antigens. It appears that granulocytes do not contain ABH antigens on their surface [57,58]. In vivo, ABO incompatible granulocytes demonstrate intravascular recoveries and survivals and normally migration into skin chambers [59]. Thus, the accumulated data indicate that ABO antigens are not present on granulocytes. Red cell antigens of the I and P_1 blood groups are on granulocytes. Rh system antigens are also not present on granulocytes.

HLA-A, B, and C (class I) antigens are on the surface of granulocytes, but the antigens are fewer in number than on lymphocytes [60]. It is possible that these HLA antigens are adsorbed onto the surface of

Table 9.13 Granuloctye alloantigens.

Antigen System	Antigen	Location	Acronym	Frequency[a] (%) Caucasians	Alleles
HNA-1	HNA-1a	FcgRIIIb	NA1	58	FCGR3B[a]1
	HNA-1b	FcgRIIIb	NA2	88	FCGR3B[a]2
	HNA-1c	FcgRIIIb	SH	5	FCGR3B[a]3
HNA-2	HNA-2a	gp50–64	NB1	97	Not defined
HNA-3	HNA-3a	gp70–95	5b	97	Not defined
HNA-4	HNA-4a	CD11b	MART	99	CD11B[a]1
HNA-5	HNA-5a	CD11a	OND	96	CD11A[a]1

Source: ISBT working party. Nomenclature of granulocyte alloantigens. Vox Sang 1999; 77:251.
[a]Calculated from data in the literature.

granulocytes [61] rather than being an integral part of the membrane, but this is not established. It appears that HLA-D/DR (Class II) antigens are not on granulocytes.

Granulocytes also contain several alloantigen systems whose tissue distribution is limited to granulocytes (Table 9.13). This was first recognized by Lalezari in studies of sera obtained from patients with unexplained neutropenia [62,63]. From his early work, Lalezari proposed the nomenclature system used for many years. The letter N stood for neutrophil, a second letter stood for the gene locus (A, B, C, etc.), and a number stood for the antigen at that locus. Thus, the antigens were named NA1, NA2, NB1, etc. The clinical significance of these antigens is described in Chapters 12 and 14. This discussion will focus on the antigens and antibodies.

The N1 antigen was the first to be discovered [62]. The antibody defining this antigen was present in the serum of a woman who delivered an infant with transient neutropenia. Her serum reacted with the neutrophils of the infant and her husband in a situation similar to red cell HDN [62,63]. A new nomenclature has been established [64] based on the glycoproteins and using nomenclature consistent with gene mapping and similar to that used for platelets. Antigens are designated HNA for human neutrophil antigen; the membrane glycoprotein is coded by a number and different antigens on that protein designated alphabetically. The HNA system is composed of five systems with seven antigens assigned to five glycoproteins.

NA-system antigens are present on the FcRIIIb portion of the Fc or gamma receptor of neutrophils [65]. The FcR molecule is a glycoprotein whose molecular weight varies with the NA phenotype because of the difference in the amount of carbohydrate side chains. The FcRIIIb is a phosphatidyl-inositol-glycan (PIG) anchored glycosylated protein. The FcRIIIb molecule has 233 amino acids and is expressed only on neutrophils, but also can be found in plasma and body fluids in a soluble

Table 9.14 Clinical situations involving granulocyte-specific antigens and antibodies.

Autoantibodies	Autoimmune neutropenia
	Drug-induced immune neutropenia
Alloantibodies	Alloimmune neonatal neutropenia
	Febrile transfusion reactions
	Transfusion-related acute lung injury
	Poor response to granulocyte transfusion

form [66]. The difference between NHA-1a and HNA-1b is four amino acids, apparently caused by one nucleotide difference in the gene. The gene is located on chromosome 1. Rare individuals lack both NA antigens and are thus NA null. These individuals lack the FcRIII molecule. The last of the HNA molecules to be characterized, HNA-3 is part of the choline transporter-like protein-2 [67, 68]. Several HNA antigens are shared with other tissues.

These granulocyte-specific antibody–antigen systems are involved in the pathophysiology of several clinical situations (Table 9.14), which will be discussed in more detail in Chapters 12 and 14. Both autoantibodies and alloantibodies have been identified, and each may cause clinical problems. These situations include autoimmune neutropenia, alloimmune neonatal neutropenia (see Chapter 12), transfusion reactions (see Chapter 14), and granulocyte transfusions (see Chapter 11). One situation in which neutrophil-specific antibodies are not important is bone marrow transplantation. We have demonstrated successful marrow engraftment of NA1-positive marrow in a patient with circulating anti-NA1 [69]. This is consistent with evidence that these antigens are not present on promyelocytes, myeloblasts, and earlier uncommitted stem cells but become expressed during myeloid maturation [70]. Thus, the granulocyte-specific antigens can be thought of as differentiation antigens specific for this cell line and that become expressed during maturation of the cell.

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10 Laboratory Detection of Blood Groups and Provision of Red Cells

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10.1 Immunologic mechanisms of red cell destruction

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Almost all red cell antibodies are either IgM or IgG (see Chapter 9). Thus, these are the immunoglobulins of importance in considering red cell destruction. Complement may or may not be involved. Red cells containing bound IgG undergo accelerated clearance through the spleen, where Fc receptors of the phagocyte bind to the IgG molecules, leading to phagocytosis of the red cell. This process does not require complement. If the IgG-coated red cell also contains bound complement components, the binding and phagocytosis are accelerated. In contrast, immune destruction of red cells coated with IgM depends on complement. IgM-coated red cells either are hemolyzed in the intravascular space or are cleared rapidly, predominantly in the liver.

Complement can cause immune destruction of red cells in either of two ways: by accelerated clearance from the intravascular space by interacting with complement receptors in the fixed macrophage system primarily of the liver, or by direct intravascular lysis due to rupture of the cell membrane by complement components. Complement can be activated by either IgG or IgM. However, the exact mechanisms that determine the ability of different red cell antibodies to activate complement are not known. IgM is much more effective than IgG in activating complement, probably because the nature of the IgM molecule places several Fc receptor sites in close proximity. However, the situation is more complex than merely the number of Fc sites in close enough proximity to activate C1. For instance, in general, anti-D (an IgG) is not effective in activating complement compared with anti-K or Jk^a (also IgG), which react with antigens that actually have fewer antigen sites than D sites on the red cell. Of IgG molecules, the subclasses of IgG1 and IgG3 are more effective in binding complement than IgG4.

The complement system involves several different proteins (complement components), the activation of which is influenced by a variety of inhibitors or proteases. Activation of C1 begins the complement cascade

sequence leading to formation of the membrane attack complex (C5–C9), which causes lysis of the red cell. Often the process does not proceed to completion of the membrane attack complex and cell lysis. However, red cells may be coated with certain complement components, which cause accelerated clearance of the red cells by interaction with complement receptors on cells of the fixed macrophage system. The first of these key steps is the activation of C3. After several activation and enzyme cleavage steps, the C3d fragment of C3 remains bound to the red cell membrane antigen–antibody complex. Red cells containing bound C3d undergo accelerated clearance in the liver by interaction with complement receptors in the fixed macrophage system. Intravascular hemolysis is the term applied to the destruction of red cells by the complement membrane attack complex. The red cell membrane is damaged, hemoglobin is released into the circulation, and the classic signs and symptoms of a hemolytic transfusion reaction occur (see Chapter 14). ABO incompatibility is the best example of red cell antibody–antigen reactions that cause this kind of hemolysis. Extravascular hemolysis is the term applied to red cell destruction caused by phagocytic cells of the fixed macrophage site. This occurs primarily in the spleen and is associated with an increase in bilirubin and its metabolites. Actually, these are arbitrary distinctions since complement may be involved in both intravascular and extravascular hemolysis and the degree or severity of hemolysis may be a factor in the symptoms and laboratory findings. For instance, red cells undergoing phagocytosis in the fixed macrophage system may be only partially engulfed and release some hemoglobin into the intravascular space, thus simulating intravascular hemolysis.

In practice, the red cell serologic tests for antibody identification and red cell compatibility are designed to enhance and speed the cell's reaction with IgM or IgG antibodies and to detect the reaction by looking for cell agglutination.

10.2 Methods of detecting red cell antibody–antigen reactions

Factors that affect agglutination

Red cell agglutination occurs in two stages: first, the antibody binds to the red cell surface; then, the antibodies interact to bring the cells in approximation, and agglutination occurs. The first stage of agglutination is affected by temperature, pH of the medium, the affinity constant of the antibody, duration of incubation, ionic strength of the medium, and antigen–antibody ratio. The second stage of agglutination is influenced by the distance between cells, the charge of molecules in the suspension, membrane deformability, membrane surface molecules, and molecular structure. Many of the practical procedures used in the daily operation of a blood bank take advantage of these factors.

Techniques to enhance red cell antibody detection

Several techniques used to enhance the antigen–antibody reaction are based on the factors known to influence antibody–antigen reactions. The techniques are (a) use of high-protein medium, usually accomplished by adding albumin, (b) use of antihuman globulin (AHG) (see Section "Antihuman globulin serum"), (c) enzyme treatment of the test red cells, (d) use of low-ionic strength solution (LISS), (e) use of polyethylene glycol (PEG), and (f) use of polybrene. Some of these techniques, such as use of AHG and LISS, can be combined.

Antihuman globulin serum

The cornerstone of red cell antibody detection is the use of AHG. AHG is prepared from the serum of rabbits immunized with human IgG or human complement, usually the C3 component. Some antiglobulin sera are a blend of monoclinal antibodies. Antiglobulin reagents have reactivity only against IgG or C3 and are called monospecific. AHG can also be prepared as a blend of these sera. This antiglobulin serum is called polyspecific. Depending on the kind of AHG used, IgG and/or C3 can be detected on the surface of red cells. The original reports and use of AHG serum involved anti-IgG [1]. During the mid-1960s, several investigators began to describe the role of complement in red cell destruction [2–4]. It was established that some red cell antibodies caused red cell destruction by activating complement and that anticomplement activity in antiglobulin reagents could predict accelerated red cell destruction [5–10]. This led to several years of research and debate as to the value of anticomplement activity in antiglobulin reagents. The rationale was that the ability to detect not only IgG but also C3 on the red cell would enhance the likelihood of finding clinically significant antibodies. However, many clinically insignificant antibodies active at cold temperatures bind complement. Since these cold antibodies are present normally in many individuals, the addition of anti-C3 to antiglobulin serum led to many clinically false-positive tests. Ultimately, a consensus developed that when anticomplement reagents are used for patient or donor antibody screening, there is a very high rate of detection of cold antibodies that are not clinically significant [11]. Thus, the practice of using anti-C3 for antibody detection for compatibility testing has been abandoned.

Although detection of C3d bound to red cells is not helpful for compatibility testing, it is helpful in the evaluation of possible immune hemolytic anemia. For instance, some patients with warm antibody-type autoimmune hemolytic anemia, cold agglutinin syndrome, and drug-induced immune hemolytic anemia may have red cells that react only with reagents containing anti-C3d activity and not with anti-IgG reagents. Thus, anti-C3 may be used in the direct antiglobulin test (DAT) to determine whether anemia or accelerated red cell destruction may be due to complement activation and/or complement-binding antibodies.

AHG serum can be used to demonstrate binding of IgG antibodies in several different techniques, including saline, high-protein, LISS, and PEG.

AHG is not used in the polybrene test because of the nature of the agglutination reactions.

Low-ionic-strength solution

At low ionic strength, the ionized groups on red cell antigen and antibody molecules become more highly charged, and the attraction between them increases. This is used as a method to enhance red cell antibody detection [12–14]. The solution used is phosphate-buffered saline with glycine added to reduce the ionic strength. Serum and red cells are incubated in the LISS at 37°C for only about 10 to 15 minutes, then the mixture is centrifuged and observed for agglutination. The LISS system is very effective in identifying red cell antibodies [15–17]. Advantages of the LISS system are increased sensitivity in detecting antibodies and the shortened incubation time, which reduces the overall time required for the compatibility test. The shortened time is an advantage when used in conjunction with the type and screen system, because on the rare occasions when blood is needed, it can be made available faster.

Polybrene

Hexadimethrine bromide or polybrene is thought to facilitate red cell agglutination by neutralizing the net negative charge between red cells and thus forming ionic bonds between the red cell and the polybrene. In the polybrene test, red cells and serum are incubated very briefly (about 1 minute) in a low-ionic-strength solution to allow antibody binding, and then polybrene is added to promote aggregation. The red cells are resuspended and, if antibody binding has occurred, the cells fail to resuspend [18]. The polybrene method is sensitive and provides results more rapidly than other methods [19, 20] but has not gained wide use. The system does not use anti-human globulin.

Polyethylene glycol

Polyethylene glycol (PEG) is a polymer that is used to displace diluent molecules in solution, effectively increasing the antibody–antigen concentration and enhancing the reaction [21, 22]. The PEG system uses a 20% solution of PEG in phosphate-buffered saline. Serum and red cells are incubated for about 15 minutes and, after washing, antiglobulin serum is added. Originally PEG was proposed only as a supplementary test to the identification of antibodies because there was a rather high false-positive rate with PEG [21]. Subsequent studies showed that PEG is more sensitive than LISS in detecting clinically significant antibodies but also had a higher false-positive rate (1.3% versus 0.1%) [22–25].

Enzymes

The antigens on test red cells can be modified by treating the cells with enzymes. The most popular enzymes are ficin, papain, and bromelin. The activity of some antigens is enhanced and others decreased (see Chapter 9). This can be especially helpful if multiple antibodies are suspected and one of the antigens is inactivated by enzymes.

Techniques for detecting red cell antigen–antibody reactions

Slide/tile typing

ABO typing can be done on slides or tiles and while this is no longer done in the United States, it is very suitable for use in resource limited situations or developing countries. Slide testing for ABO blood type is performed using either whole blood or suspended red cells, depending on manufacturer instructions. A single drop of anti-A is placed on a clean slide; the same is done with a single drop of anti-B and anti-A, B. A drop of well-mixed red cell suspension is added to each slide. The reagents and red cells are mixed thoroughly using a clean applicator stick. The slide is kept away from heat and gently tilted, and after 2 minutes, strong agglutination of red cells in the presence of any reagent indicates a positive result, while a smooth suspension indicates a negative result.

Tube tests

Traditionally, red cell testing was being carried out in tubes. The red cell serum mixture is observed macroscopically under strong light, usually by tipping the tube over a mirror. If agglutination is not observed using this macroscopic method, some technologists prefer to pour the cell suspension onto a slide and observe the mixture under a microscope to find very weak reactions. The largest study [26] of this issue involved more than 200,000 antibody detection tests in patients. Antibodies that necessitated antigen-typed red cells were found in only seven patients (0.02%). Therefore, microscopic observation of antibody detection tests is not necessary.

Solid-phase tests

Solid-phase assays [27–30] are usually done in microtiter plates. The target antigen is fixed to the wells of the plate and the test serum or plasma is added. Often low-ionic-strength solution is also used to enhance the antibody–antigen reaction. After incubation, the plates are washed. Reagent red cells coated with AHG are added, and if antibody was present in the test serum, the reagent red cells will adhere to the plate. The reaction can be read by a technologist or an automated plate reader. This system can be used for ABO and Rh typing and for antibody detection or identification, depending on the reagent red cells used to coat the plates.

Gel test

The gel test [31, 32] is based on the principle of size exclusion. The gel is dextran-acrylamide particles. The tubes come prefilled with gel and the test serum and/or reagent red cells are combined at the top of the tube. The reaction mixture is incubated and then centrifuged. The centrifugation drives the red cells into the gel. If antibody is present, large red cell agglutinates form and the red cells are trapped at the top of the gel column. The gel system can be used for ABO and Rh typing, red cell antibody detection, identification, and crossmatching.

Affinity column

Affinity columns [33] take advantage of the immunologic binding of IgG to staphylococcal protein G. The columns are composed of protein G bound to agarose. Test plasma or serum is combined with reagent red cells at the top of the tube but prevented from entering the agarose. The reaction mixture is incubated and then centrifuged. If antibody binding to the red cells has occurred, the IgG-coated red cells are trapped by the G protein at the top of the column. If agglutination has not occurred, the red cells not coated with IgG travel through the column and are found at the bottom.

10.3 Direct antiglobulin (coombs) test

The DAT demonstrates antibody coating of red cells. Washed red cells from a patient or donor are incubated with AHG, washed, centrifuged, and observed for agglutination. Antibody–antigen enhancement strategies are not used because the test is intended to determine whether antibody has been bound to the red cells in vivo. Monospecific or polyspecific reagents can be used. Monospecific IgG is most commonly used, although if a polyspecific reagent is used, cells that react positively are then tested with monospecific reagents to determine whether IgG or C3 is bound. Traditionally, the DAT was done in a test tube but as new red cell antibody detection systems have been developed, they are sometimes used for the DAT. The gel microcolumn has been reported to be more [34, 35] or less [36] sensitive than the tube technique. It seems wisest, as advised by Dittmar et al. [34], to use a second method if a patient with suspected immune hemolytic anemia has a negative DAT initially. The DAT is positive in a wide variety of situations (Table 10.1). The role of the DAT in autoimmune hemolytic anemia is discussed in Chapter 12, hemolytic disease of the newborn (HDN) later in this chapter, normal individuals in Chapter 8, and hematopoietic stem cell transplants in Chapter 12.

10.4 Red cell compatibility testing

The term compatibility or pretransfusion testing refers to all procedures involved in providing for the patient blood products that "will have

Table 10.1 Situations in which the direct antiglobulin test may be positive.

Autoimmune hemolytic anemia
Drug-induced immune hemolytic anemia
Hemolytic disease of the newborn
Recently transfused patients who have made alloantibodies to donor red cells
Patients who have undergone hematopoietic stem cell transplantation
Patients with various autoimmune diseases
Patients with some hematologic malignancies
Patients who have received an ABO-incompatible organ transplant
Some normal individuals

Table 10.2 Key steps in compatibility testing.

ABO and Rh typing of donor red cells
Testing of donor serum for red cell antibodies
Identifying the patient, acquiring, and labeling patient blood sample
Reviewing patient and donor records
ABO and Rh testing of the recipient
Testing the recipient's serum for red cell antibodies
Identifying recipient red cell antibody if present
Selecting the proper blood component
Carrying out the major crossmatch
Labeling the blood component and completing all records

Source: Adapted from Shulman IA, Petz LD. Red cell compatibility testing: clinical significance and laboratory methods. In: Petz L, Swisher S, Kleinman S, Spence R, Strauss R, eds. Clinical Practice of Transfusion Medicine. New York: Churchill Livingstone, 1996.

acceptable survival and will not cause clinically significant destruction of the recipient's own red cells" [37]. The crossmatch is only one part of the compatibility or pretransfusion test (Table 10.2). All steps from the collection of the blood sample from the patient to the release of the blood component from the blood bank are important in providing a safe and effective transfusion.

For ABO typing, the methods involve incubation of red cells and antisera at room temperature for about 5 minutes, centrifugation, and observation of the cell suspension for agglutination. This can be done because the ABO antibodies react almost immediately at room temperature. For antibody detection, more complex testing is necessary. Because antibodies in patients are more dangerous clinically, the antibody detection techniques used in testing patients' serum are usually different from those used for screening donor sera. For donor antibody detection (screening), reagent red cells are usually pooled, and no additives or enhancement reagents are used except for AHG (see Chapter 8). For patient testing, one of the enhancing methods is often used. For instance, testing might be done using enzyme-treated reagent red cells, or the media might be enhanced using albumin, LISS, or PEG or polybrene [38]. The increments improve the likelihood of detecting weak antibodies in the patient.

The results of the antibody detection test on the patient determine the crossmatching strategy to be used. If no antibody is detected, the crossmatch need involve only a method that will detect ABO incompatibility between the donor and recipient in an effort to avoid an ABO-incompatible transfusion. The results of the antibody detection test are considered valid for 3 months unless the patient has an experience, such as pregnancy or transfusion, that could stimulate red cell antibody formation. If an antibody is detected, the antibody should be identified (see below). Once the antibody is identified, the donor units can be typed for the corresponding antigen, and those units crossmatched to ensure compatibility between donor and recipient. The antibody identification

Table 10.3 Indications for repeat identification of red cell antibodies.

Clinical evidence of a hemolytic transfusion reaction
Change in reactivity in antibody screening test
Incompatibility with donor unit antigen negative for patient's antibody
Change in reactivity of DAT or auto control
Increased need for red cell transfusion
Icteric serum

Source: Adapted from Harris, T. Repeating antibody identification, AABB News 2000; Oct:4, 26.

need not be repeated on subsequent samples unless there is clinical or serological evidence of new antibody formation [37]. Situations that suggest the need to reidentify the antibody are listed in Table 10.3.

Positive identification of recipient and blood sample

The most common cause of a fatal hemolytic transfusion reaction is the administration of an ABO-incompatible unit of red cells. This often happens because of errors in collecting the original blood specimen from the patient or in administering the unit of blood to the wrong patient (see Chapters 13 and 14). Because of this, the acquisition of the blood sample for compatibility testing is extremely important. The procedures and techniques for obtaining the blood sample are described in Chapter 13. Labeling of the tubes to be used for the blood sample should occur at the patient's bedside, and great care should be taken to ensure that the blood sample is being obtained from the patient whose identity is being placed on the tube. This is done by asking patients to confirm their identity; for patients who are unable to communicate, each hospital should have a procedure to ensure the proper identity of the patient from whom the sample is being obtained.

Review of transfusion service records for results of previous testing of samples from the recipient

When the request for transfusion and blood sample is received in the blood bank laboratory, the first step is a review of blood bank records to determine whether any blood samples have been received previously and any laboratory work carried out. If so, the patient's ABO and Rh blood types should be available in the records as well as the results of the antibody screening test. If the antibody screening test was positive, the identification of the antibody will also be available. These results do not substitute for proper laboratory testing of the new blood specimen but are used for comparison with the new results. If there are discrepancies in the ABO or Rh type, this must be resolved before blood can be released for transfusion. These discrepancies suggest a mix-up or misidentification of either the earlier or the present blood specimen. In one French study, ABO discrepancies were found at a rate of one in 3400 blood samples. Most of these discrepancies were due to collection of blood from the wrong patient [39]. Some hospitals now require that if there is no record of a previous red

cell type, a second blood sample be obtained from the patient to confirm the ABO and Rh type before blood is released for transfusion.

Antibodies present in previous blood samples may no longer be found because of natural changes in the strength of antibody activity. However, knowledge of antibodies previously present is important, because those antibodies could be expected to reappear quickly after transfusion of antigen-positive red cells. Thus, even though the antibody may no longer be present, the patient should receive red cells that lack the corresponding antigen to avoid a delayed hemolytic transfusion reaction (see Chapter 14).

ABO and Rh typing

Many clinical conditions and diseases may make interpretation of the results of ABO and Rh typing difficult. Careful attention to detail and the proper use and interpretation of controls is essential. The ABO and Rh type of the patient is confirmed on each blood sample used for pretransfusion testing to increase the safety of the transfusion by ensuring that the sample is from the correct patient. The ABO and Rh type of the donor blood will have been confirmed when the hospital received the blood from its regional blood supplier, or by duplicate typing if the hospital collects its own blood supply.

Selection of blood components of appropriate ABO and Rh types

For routine transfusions, red cells are selected that are identical with those of the patient for the ABO and Rh(D) antigens. In some situations, such as massive bleeding or an emergent need for transfusion, red cells that are not ABO and Rh(D) identical may be used (see Chapter 12). When Rh-negative patients experience massive bleeding, it may be necessary to use Rh(D)-positive blood because of unavailability of Rh-negative red cells. This decision should be made by consultation between the blood bank physician and the attending physician. If necessary, Rh-negative red cells can be given to Rh-positive patients, although this is not usually done due to the need to conserve Rh-negative red cells for Rh-negative patients. Since Rh-negative red cells lack the D antigen, there will be no unusual adverse effect on the Rh-positive patient.

It may also be necessary to switch ABO types. When blood of a different ABO type is used, it is used as red cells, not whole blood, to avoid problems due to transfusion of antibodies contained in the plasma. Table 10.4 indicates the different ABO types of donor blood that can be used. Despite the small amount of plasma in the red cell unit, hemolysis due to passive transfusion of antibody in these units can occur. However, this is extremely rare, and the remote likelihood of such a problem should not interfere with making red cells available rapidly in urgent situations.

Antibody detection (screening) test (indirect antiglobulin or indirect coombs test)

The antibody detection or screening process for testing donor blood is described in Chapter 8. Detection of antibodies in the recipient is even

Table 10.4 ABO antigens, antibodies, and donors suitable for transfusion to patients of different ABO types.

Gene	ABH antigen	ABO type	Antibody present	Preferable donor	Other acceptable donors[a]
A	A	A	B	A	O
B	B	B	A	B	O
H	H	O	A & B	O	None
A & B	A & B AB	None	AB	A or B or	O[b]

[a] If blood is used as red blood cells.
[b] Group A is preferable.

more important, because antibodies in the patient are generally more dangerous than those in the donor. The antibody to cell or antigen ratio is higher when the antibody is in the patient because the donor antibody, if present, is diluted in the patient's entire blood volume. In the antibody screening test, the patient's serum is reacted with red cells from usually three (some laboratories use only two) normal individuals selected to provide cells that contain antigens reactive with all of the common, clinically significant antibodies. The cells are usually purchased commercially and are subject to US Food and Drug Administration (FDA) requirements for the antigens they contain and their strength of reactivity. The test is usually done in tubes, although an increasing number of transfusion service laboratories use the gel technique using automation. The conditions of this test usually involve incubation of the patient's serum with test red cells suspended in LISS, saline or albumin followed by washing the red cells, adding AHG, centrifuging, and looking for agglutination. There is extensive literature describing the optimum technique for detecting different antibodies. The development of cardiac surgery involving hypothermia occurred during the years when new blood group antigens and antibodies were frequently being discovered. Some of these antibodies were optimally active at temperatures below normal body temperature (see Chapter 9). Thus, techniques were added to routine pretransfusion and compatibility testing to detect antibodies reactive in the cold. However, as more experience was gained, it became clear that it is not necessary to avoid cold-reacting antibodies for patients undergoing hypothermia during routine surgery [40]. In addition, almost all of the cold antibodies detected on routine testing were not clinically significant, and this test procedure was gradually eliminated [41]. Some of the other methods that may be used include treating the red cells with enzymes, extending the length of incubation, changing the serum to cell ratio, altering the incubation temperature, or suspending the red cells in low-ionic-strength solution and using chemicals such as polybrene to enhance agglutination. Some of these techniques are used routinely by some blood banks; others are suitable for use only in reference laboratories for investigation of certain antibodies. The tests can be carried out in any of the tube, gel, solid-phase, or affinity systems described above. If the
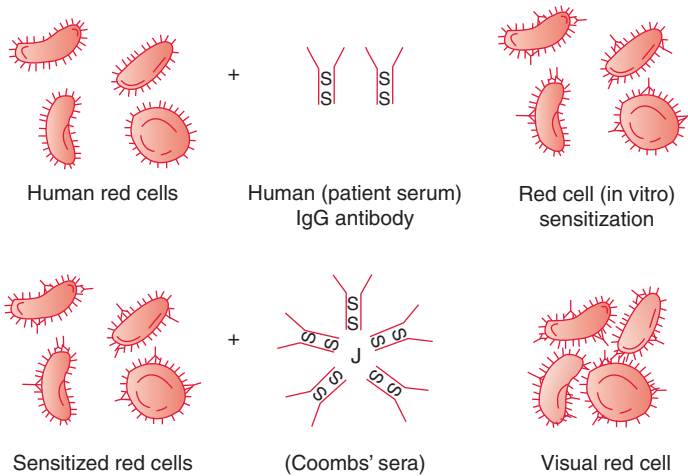
Figure 10.1 Schematic illustration of crossmatch using antihuman globulin. (Adapted from Harmening D. Modern blood banking and transfusion practices. Philadelphia, FA Davis, 1989.)

patient's serum contains an IgG antibody that reacts with an antigen on the red cells used in antibody screening tests, this will be detected using the antihuman IgG (Figure 10.1). This is an extremely important test, since most clinically significant red cell antibodies are IgGs.

If an antibody is detected in the patient's serum, the antibody should be identified and red cells selected that lack the corresponding antigen if the antibody is clinically significant (see Chapter 9). This approach is preferable to selecting units of red cells that are compatible in a crossmatch without identifying the antibody. The antibody in the patient's serum may be too weak to react with red cells heterozygous for the antigen (the so-called dosage phenomenon) and thus the crossmatch will appear to be compatible. However, transfusion of the red cells heterozygous for the antigen may result in accelerated destruction of the cells. Rarely, reactivity may occur when serum but not when plasma is used. This does not appear to represent true red cell antibody reactivity and is not clinically significant [42]. Rarely, hemolysis may occur when the antibody screening test is negative (Table 10.5).

The crossmatch

If the antibody screening/detection test is negative, the crossmatch technique can be limited to one that will detect ABO incompatibility. If the antibody screening test is positive, a full or more extensive technique must be used.

Full crossmatch

The crossmatch involves testing the donor's red cells against the patient's serum. In the past, this was done in several different "phases" such as room temperature, after incubation at 37°C, and after the addition of

Table 10.5 Situations in which hemolysis can occur when the antibody screening test is negative.

Antibody to low-frequency antigen not on screening cells
Passive transfer of donor antibody
Incorrect blood administered
Infusion using hypotonic solutions
Improperly stored unit
Improperly thawed frozen red cells
Bacterial contamination of unit
Underlying sepsis of patient
Antibody undetectable by existing laboratory methods

antiglobulin serum. This group of methods was very effective in detecting all clinically significant antibodies. Albumin was preferable to a saline suspension because using saline and antiglobulin weak antibodies, poorly reactive red cells, or the prozone phenomenon resulted in false-negative tests. Experience then gradually established that the room temperature phase identified many antibodies (reactivity) that were not clinically significant. Over a period of several years, the room temperature phase of testing was eliminated and the saline suspension test, along with an antiglobulin phase, was used to detect all clinically significant antibodies.

In the full major crossmatch, the recipient's serum is reacted with the intended donor's red cells, usually using a technique similar to that used in the antibody detection test. This involves incubation at 37°C and the use of AHG. Both antiglobulin reagents (polyspecific containing anti-IgG and anti-C3, or monospecific containing anti-IgG, only) are in routine use, but the detection of cell-bound C3d may lead to false-positive results, and some workers prefer to use the anti-IgG reagent. Various agents or procedural modifications described previously may be used to attempt to enhance or speed antibody–antigen reactions.

Abbreviated (ABO) crossmatch

At about the same time as these methods were evolving, interest developed in using the antibody screening test more extensively instead of the crossmatch between patient's serum and donor cells [43–46]. Experience established that antibody screening could replace the antiglobulin phase of the crossmatch for patients in whom no antibody is detected in the antibody screening procedure [47, 48] and a complete crossmatch is not necessary [49]. Thus, it is increasingly common to carry out a simplified crossmatch that confirms ABO compatibility if the patient has a negative antibody detection test and has not been pregnant or transfused within the preceding 3 months [37]. Thus, for patients with no antibodies detected in the antibody screening test, the "crossmatch" is used to detect ABO incompatibility. This can be done with a simplified method involving a saline suspension medium and a short incubation of about 5 minutes [47, 48]. It is important to allow this incubation period so that antibodies have time to react and to avoid false-negative tests due to the prozone

phenomenon. This test is usually done with the tube or gel method but can be done as a slide or a simple rapid slide polybrene method for pretransfusion compatibility testing can be done without a centrifuge and may be an acceptable method for compatibility testing in developing countries [38].

The abbreviated crossmatch will not completely prevent the rare transfusion of an incompatible unit, since some blood group antigens of low frequency will not be present on the red cells used for antibody screening tests. However, this occurs infrequently [50, 51] and many of these incompatibilities can be detected in the abbreviated (ABO) crossmatch [51]. When incompatibility frequencies are considered, 99.99% of patients who have a negative antibody screening test would receive compatible red cells [50]. Several other studies report relevant data. Alexander and Henry [52] found seven hemolytic transfusion reactions in 1.5 million red cell transfusions (1/214,000) due to antibodies missed in the screening test. Cordle et al. [53] found that one positive crossmatch in 3380 serum samples with a negative antibody screening test. The antibody was anti Kp^a. This was thought to potentially cause a transfusion reaction, but not one of "serious consequence to an adult patient." Shulman et al. [49] reported no acute hemolytic transfusion reactions in 19,818 patients with a negative antibody screening test when they were transfused using an abbreviated crossmatch, and Heisto [54] found one antibody in 73,407 crossmatches of screening negative sera. Therefore, the standard approach has become to focus on the antibody screening procedure and, when it is negative, carry out an abbreviated crossmatch designed to detect ABO incompatibility. For this approach to be optimally effective in reducing costs, the patient's blood sample should be sent to the blood bank the day (or more) before surgery because the collection time of the sample is related to the likelihood of delayed surgery [55].

Minor crossmatch

In the minor crossmatch, the donor's serum is reacted with the patient's red cells. This test was intended to detect antibodies in the donor that might cause hemolysis of the patient's red cells. Although a few such cases have been reported, this is very rare. The situation is now approached by carrying out an antibody detection test on the donor's serum at the time of donor blood processing. If donor antibodies are present, the unit is either not used or the plasma is removed. Thus, the minor crossmatch is not necessary and is no longer used.

Computer crossmatch

The next phase in the evolution of compatibility testing was the recognition that confirmation of ABO compatibility could be accomplished by methods other than laboratory tests. This led to the proposal for a "computer crossmatch" [56]. In the "computer crossmatch" the laboratory test for ABO compatibility is replaced by a computer check of the records of the donor and patient [57, 58]. The ABO type of the patient must have been done at least twice, once on the present sample,

and the computer must contain all pertinent data about the donor unit and the patient. Safwenberg et al. [59] reported on 12 years of experience with computer crossmatches. The antibody screening methods evolved during the 12 years of data gathering. Initially, the test used enzyme (bromelin)-treated reagent red cells, then an antiglobulin-phase tube test but no enzyme, and then an antiglobulin test carried out in a gel system. This represented a shift in emphasis of the screening procedure over the years from maximum sensitivity (with many false-positive reactions) to more specificity. Four different red cell suspensions were used as screening cells. The rate of alloantibody detection was about 1%. Combining all three of these antibody screening methods, the computer crossmatch system with antibody screening allowed 90% of the units to be released without a further laboratory crossmatch. There were no cases of hemolytic transfusion reactions due to failure of the system. The authors believe that the value of this approach is that it allows antibody screening to be carried out under more optimum conditions than in the crossmatch. The drawback is that the screening cells must be selected to contain the proper spectrum of antigens so that clinically significant antibodies will be detected. As the authors point out, this system will not detect a mislabeled unit, as happened once in their experience. Thus, the failure rate of the computer crossmatch in this study was 1/257,400 units [59].

Labeling and issue of the appropriate blood products

Records of all laboratory tests should be made at the time the laboratory work is being done. At the completion of all laboratory testing, the records should be complete and the unit of red cells found to be compatible can be labeled and prepared for release for transfusion. Most blood banks today use bar code labeling systems, but whatever the nature of the label, a tag of some sort is attached to the unit. This "crossmatch tag" contains the patient's name, identification number, blood type, and the blood type and identification of the unit of red cells found to be compatible. Also, the tag contains the statement that this unit is compatible and suitable for transfusion to the specified recipient. Thus, the transfusionist can review the patient's identification, the identity of the unit of red cells, and the information on the crossmatch tag to ensure that the proper unit of red cells is being administered to the patient (see Chapter 13).

10.5 Red cell antibody identification

If the antibody screening test is positive, the antibody should be identified so that antigen-negative red cells can be selected for the crossmatch. Antibody identification is done using reagent red cells from several individuals (usually eight) selected to contain antigens corresponding to the clinically significant antibodies (Figure 10.2). This group of reagent red cells is called a "panel." The technique used for identifying the antibody may be the same as that used for the antibody screening, or an alternative technique may be used. An alternative technique might be used because

CAPTURE-R READY-ID EXTEND I
(D-Positive Panel)
Master List

NAME _____
NO. _____
INSTITUTION _____
BLOOD GROUP _____
ANTIBODY IDENTITY _____
TECH _____ DATE _____

CELL	Special Type	Donor	D	C	c	E	e	f	V	Cw	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Xg^a	CELL
1		R1wR1 B6201	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	0	+	+	1
2		R1R1 B7068	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	0	+	0	0	+	+	0	+	+	+	0	+	+	2
3		R1R1 B6882	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	0	+	+	0	+	+	3
4		R1R1 B7450	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	+	4
5		R1R1 B1923	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	0	5
6	Yt(b+)	R1R1 B5986	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	+	0	0	+	+	6
7		RzR1 A3814	+	+	0	+	+	0	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	+	7
8	Co(b+), Yt(b+)	R2R2 C1664	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	8
9		R2R2 C2688	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	0	+	0	+	+	9
10	Bg(a+)	R2R2 C4638	+	0	+	+	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	10
11	Co(b+)	R2R2 C1689	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	+	+	0	+	+	0	0	+	+	+	0	11
12		R2R2 C4700	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	0	+	0	+	0	12
13		R2R2 C4703	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	13
14		R2r J332	+	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	+	0	+	0	14
15		POSITIVE CONTROL	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	PC
16		NEGATIVE CONTROL	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	NC

Rh-Hr | Kell | Duffy | Kidd | Lewis | P | MN | Lutheran | Xg

PATIENT'S SERUM — ANTIBODY SCREEN — LOT

| I |
| II |
| III |
| IV |

PATIENTS TEST RESULTS

NOTES:

^N The f antigen status may have been determined presumptively based on Rh-Hr phenotype.

An antigen designated with a 'w' represents weakened expression of the antigen that may or may not react with **all** examples of the corresponding antibody.

All cells are positive for I, Ge, Yta⁺ Tja, Vel, Coaᵃ, Dib and negative for Mg, Vw, Dia, and Wra except where noted.

* Indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.

Figure 10.2 Example of a panel of reagent red cells used for antibody identification. Copyright material used with the permission of Immucor, Inc.

experience has established that method to be most effective locally, or because the screening test suggested a particular antibody specificity that is best demonstrated by a particular technique. It may be necessary to test the patient's serum against more than one panel or against sets of red cells especially selected to possess or lack certain antigens. In addition, since certain specific antibody–antigen reactions are known to be enhanced or inactivated by different conditions, these special tests may use techniques different from those used for antibody detection or the initial panel test. Examples of some of these special techniques are given below.

Chemical modification of test red cells

Chloroquine can be used to elute IgG from red cells [60]. This is of value in patients with a positive DAT. The IgG can be removed, making it possible to type the patient's red cells, which provides information about which antibodies the patient could produce. Test red cells can also be treated with dithiothreitol (DTT) to inactivate some antigens [61]. DTT acts by disrupting sulfhydryl bonds, thus altering the tertiary structure of some antigens and rendering them less reactive. A third method of chemically treating red cells involves a reagent referred to as ZZAP [62]. This combines the enzyme papain with DTT to inactivate Kell, MNSs, Duffy, Gerbich, and most LW, Cartwright, Dombrock, and Knops antigens. This is probably a more effective way to inactivate red cell antigens than DTT alone. These strategies to inactivate certain known red cell antigens and then repeat the testing of the serum containing the unknown antibody can be a very helpful strategy for identifying red cell antibodies.

Enzymes and enhancement media

The use of enzymes to increase or decrease the reactivity of certain antigens and the use of enhancement media such as LISS, PEG, and polybrene have been described previously.

Neutralizing or inhibitor substances

Substances can be obtained commercially that inhibit certain antibody–antigen reactions. In trying to solve complex antibody problems, it may be easier to neutralize the antibody than to find a group of red cell suspensions that lack the suspected antigen. Examples of antigens for which this strategy can be used are P1, Lewis, and Chido/Rogers.

Sulfhydryl reagents for distinguishing IgG from IgM antibodies

Sulfhydryl reagents can be used to distinguish IgG from IgM antibodies [63]. DTT is most commonly used. The DTT cleaves the disulfide bonds between the subunit chains of IgM but does not affect the bonds between the monomers of IgG. This treatment of a serum containing a red cell antibody with DTT will inactivate an IgM but not an IgG antibody. This can be used conveniently in antibody identification.

Absorption

Absorption is of value primarily to remove cold-reactive antibodies that interfere with antibody identification. Absorption can be done using autologous red cells to remove cold-reactive antibody and the serum then tested at 37°C to detect warm-active alloantibodies. In patients suspected of having multiple alloantibodies, absorption can be done using allogeneic red cells selected to contain or lack specific combinations of antigens.

Elution

Elution is the process of removing bound antibody (usually IgG) from the red cell surface. This is done on red cells of patients who have a positive DAT. The recovered antibody in the eluate can then be identified using the same techniques that are used to test serum. Removing (eluting) bound antibody also makes it possible to type the patient's red cells to determine which antibodies the patient could form. There are several elution techniques including heat, cold acid, glycine HCl, Lui freeze/thaw, acid, digitonin, and gentle heat.

10.6 Strategies for making red cells available for transfusion

Blood availability

Red cell transfusions are used for replacement in chronic anemia and either controlled or uncontrolled blood loss. Transfusions for chronic anemia and controlled blood loss can be planned, and all of the steps described above can be carried out in an efficient, well-organized manner (Table 10.6). Most red cell transfusions are provided to patients who are relatively stable; the process is uneventful and is performed at a reasonable

Table 10.6 Strategy for providing blood.

Little blood loss, no expected transfusion
  Type and screen
  No antibody
    No crossmatch
    No blood set aside
  Antibody present
    Complete crossmatch
    Blood set aside
Large blood loss, transfusion expected
  Type and screen
  No antibody
    Crossmatch—ABO compatibility
    Blood set aside
  Antibody present
    Crossmatch—complete
    Blood set aside

Table 10.7 Approximate time required to provide compatible RBCs in different situations.

Emergency method	Minutes
Complete compatibility test	60[a]
ABO, Rh type, and antibody screen	
Crossmatch	
Emergency crossmatch—previous sample	10
ABO crossmatch	
Emergency crossmatch—no previous sample	15
No crossmatch	10
ABO type specific	
No crossmatch	5
Universal donor	

[a]If antibody screen negative; if antibody screen is positive, time may be 120 minutes or much longer.

pace. The ABO and Rh typing, red cell antibody detection, and subsequent crossmatching, if the patient does not have any unexpected red cell antibodies, can be expected to take approximately 1 hour if other more urgent laboratory work does not interfere (Table 10.7). Thus, at best, red cells could be expected to be available within about an hour after receipt of the specimen and request forms in the blood bank. When patients experience acute blood loss, initial management is with intravenous fluids to maintain the blood volume (see Chapter 12), but there may be an urgent need for red cell replacement. For these situations, it is essential that the blood bank have policies, procedures, and systems in place to make red cells available quickly and safely. There are several strategies that can be used to provide blood quickly. Each different strategy that shortens the time required to make red cells available involves eliminating some of the standard testing procedures. Because there is some possibility for increased risk of error or failure to detect red cell incompatibility, effective communication between the patient care team and the blood bank staff is essential. In this way, sound judgments can be made to balance the patient's clinical needs for urgent transfusion against the drawbacks of abbreviating the pretransfusion testing. The following sections describe the approaches to providing red cells under different conditions ranging from planned elective transfusion to the most urgent, life-threatening situations. These different situations, the time required and the trade-offs in patient safety are discussed below. Many hospitals are establishing "massive transfusion" protocols that make large amounts of red cells, plasma, and platelets available quickly (Chapter 12).

Standard or maximum surgical blood ordering

The amount of blood a hospital must maintain in its inventory is based on the amount ordered to be set aside (crossmatched), not the amount used. Thus, it is important to avoid ordering and sequestering excessive amounts

of blood. To reduce this possibility, a structured approach to providing blood for elective surgery is used. Analysis of each hospital's blood usage for particular procedures is done to develop baseline data for that hospital. Alternatively, data is available from the literature describing the amount of expected blood use for different procedures. Based on these data, a standard or maximum amount of blood to be requested is defined for each type of surgical procedure [64–67]. This becomes the routine amount of blood that is crossmatched and set aside for a particular patient undergoing that procedure. This minimizes blood wastage by avoiding over-ordering blood and is referred to as the maximum or standard surgical blood order program. For procedures in which blood is rarely used, blood is not crossmatched and set aside in advance. Instead the "type and screen" procedure is used.

Type and screen

For surgical procedures or other situations in which blood is used rarely, if at all, blood is not crossmatched and sequestered for a particular patient, but instead the "type and screen" procedure is used [43]. In the type and screen, the patient's ABO and Rh blood types are determined and an antibody detection (screening) test is done. If no unexpected red cell antibodies are present and the patient is scheduled for a procedure in which blood is rarely used, no blood is set aside and no crossmatching is carried out. If the rare situation arises in which the patient requires a red cell transfusion, red cells are provided either using the ABO crossmatch (which requires approximately 15 minutes) or, in an emergency, red cells ABO identical with the patient can be released without a crossmatch (Table 10.6). If an antibody is found in the antibody detection test, then the antibody is identified and the crossmatch technique to be used for that patient is defined. Often blood will be crossmatched and held for patients with complex antibodies in circumstances where the standard surgical blood request would not warrant this. Even though preparing blood for these patients involves additional work, it avoids the potential difficulties in making blood available quickly for patients who have red cell antibodies and for whom it may be difficult to locate red cells lacking the antigen in question and compatible in a crossmatch.

Emergency "crossmatch"

This is the same as the abbreviated or ABO crossmatch. When blood is needed urgently and a blood sample from the patient has been obtained earlier and is in the blood bank, the blood type and antibody screening result may already be known from testing of that sample. If no red cell antibody was found in the screening procedure, a unit of red cells that is ABO identical with the patient can be selected and the abbreviated or ABO compatibility crossmatch can be performed in about 10 minutes.

If a blood specimen had not been obtained previously and the blood bank must determine the blood type on a new sample, this can be done within about 5 minutes and the proper ABO type unit selected and crossmatched as described above. This adds about 5 minutes to the time

required, or a total of about 15 minutes for release of blood on an
emergency basis. However, in this situation an antibody screening test is
not performed, and so it is possible that a red cell antibody other than
ABO will be present and incompatible red cells could be transfused. When
the ABO crossmatch is being set up in the laboratory, the antibody
screening test will also be set. Often the antibody screening test result is
known by the time the blood is released from the blood bank and
transported to the patient's bedside. If an antibody is present, the blood
bank technologist can telephone the patient care unit, and this information
can be taken into consideration by the physician in deciding whether to
continue the transfusion. Even when the antibody screening test is positive,
it may be appropriate to continue the transfusion because of the patient's
critical condition.

Uncrossmatched red cells

Rarely in patients with trauma, unexpected massive intraoperative
hemorrhage, or ruptured aortic aneurysm, red cells are needed urgently
and it is not thought to be acceptable to wait approximately 10–15 minutes
to obtain red cells that are ABO identical with the patient and have
undergone an ABO crossmatch. In these situations, uncrossmatched red
cells can be given. Whenever a crossmatch is not done, group O red cells
are used. Usually Rh-negative red cells are selected, because the
combination of O-negative red cells should avoid a hemolytic transfusion
reaction to the most common clinically important red cell antibodies:
anti-A or anti-B and anti-D. In the past, many hospitals attempted to
maintain a small stock of O-negative red cells in the emergency
department so that a transfusion could be started immediately. This
practice is not advised. The techniques for managing acute blood loss are
very effective, the wastage of these red cells is very high, and it is not
practical to obtain an adequate supply of O-negative red cells for storage in
emergency departments. Uncrossmatched red cells can be made available
quickly and their use does not seem to be associated with increased risk,
although the need for uncrossmatched red cells is often overstated [68, 69].

Factors that influence blood availability

In addition to the time necessary in the laboratory, there are practical
operational factors that influence the speed with which red cells can be
made available. These are: (a) the distance of the blood bank from the site
where the red cells are needed (usually the operating rooms), (b) the
transportation system to be used, and (c) the time of day that the need
arises. Obviously the time to provide red cells should be much shorter if
the blood bank is nearby, but this can be influenced by the transportation
system. If human "runners" are used, they may not be immediately
available and it is not usually advisable for the blood bank staff to leave the
laboratory to take blood to the patient care area. A mechanical transport
system should be immediately available but it must be dependable. If the
emergency occurs at night or on a weekend, there may not be adequate
staff to respond, thus delaying red cell availability. In one study [70] of

466 hospitals, the average time from urgent request to arrival of red cells in the operating room was 34 minutes with most of the time taken from request to release of blood from the blood bank. Factors associated with more rapid release were having lists of patients and procedures in the blood bank, adequate blood specimens in the blood bank, and completed type and screen procedures.

10.7 Approach to the patient with an incompatible crossmatch

In compatibility testing when there is reactivity in the antibody detection test, serologic studies should be carried out to determine whether a red cell antibody is present [71]. If a clinically significant antibody is identified, donor red cells typed and found to be negative for the corresponding antigen should be compatible when crossmatched. The blood bank staff will carry out these procedures and provide compatible blood, although additional time and blood samples may be necessary. For several reasons, this approach of identifying the antibody and selecting known antigen-negative red cells is preferable to merely crossmatching until compatible red cells are found. The panels used for red cell antibody identification do not represent random donors, and thus the antibody may react with a higher proportion of donors than the portion of reactive panel cells, making it difficult to obtain compatible red cells. Some antibodies react in vitro only with red cells homozygous for the antigen but cause in vivo destruction of heterozygous red cells; and many sera that react in vitro do not signify potential in vivo red cell destruction. Thus, knowledge of the specificity of the red cell antibody and the character of anti-red cell reactivity improves the safety of the transfusion.

Some patients' serum reacts with many or even all of the donors tested. In this situation it is necessary to determine whether the patient has autoimmune hemolytic anemia. This is usually indicated by a positive DAT. The patient may or may not be anemic. The selection of blood for patients with autoimmune hemolytic anemia is discussed later. If the patient has a negative DAT and does not have autoimmune hemolytic anemia but the serum reacts with all donors' red cells tested, it must be determined whether an alloantibody is present against a high-frequency antigen or whether the reactivity is due to other nonred cell blood group factors. Examples of nonred cell blood group factors are abnormal proteins in the patient's plasma, high titers of normal cold agglutinins, and reactivity with test materials such as preservatives or antibiotics used in the test red cell suspension. If it is determined that the patient does have a high-frequency alloantibody, the clinical significance of the antibody determines whether it is preferable to obtain antigen-negative red cells. For instance, this is unnecessary for many antibodies that do not cause transfusion reactions, hemolysis, or accelerated clearance of red cells. If the alloantibody is expected to be clinically significant, most blood banks have local files of rare donors and, if necessary, national rare donor registries of

the American Red Cross or American Association of Blood Banks can be contacted. In patients with a clinically significant high-frequency alloantibody, the decision as to whether to delay transfusion to obtain compatible antigen-negative red cells will depend on the patient's condition. If the patient's condition does not allow delay, an in vivo crossmatch can be done (see below). In situations where no specific antibody has been identified, considerable emphasis has been placed on selecting the least incompatible donor units for transfusion. While it is unlikely that this is detrimental, there is no evidence that in these situations, red cells less reactive in vitro will have better survival in vivo.

In vivo red cell compatibility testing

Occasionally, compatible donor red cells cannot be found either because the patient's serum contains an autoantibody that reacts with all donors' red cells or the serum contains an alloantibody against an extremely high-frequency antigen and cells lacking the antigen are not available. In the past, occasionally an "in vivo crossmatch" was done. This involved labeling with ^{51}Cr a small volume (usually 5 mL or less) of the red cells to be transfused, injecting them, and determining the percentage that survive 24 hours later. In general, patients do not experience an acute hemolytic reaction if at least 85% of the donor red cells survive for 24 hours [72]. However, the 85% value should not be used as an arbitrary indication of a safe transfusion. Transfused red cells with lower 24-hour survivals in the in vivo crossmatch may have a better survival when the larger-volume transfusion is given, and occasionally patients may experience severe hemolysis despite in vivo crossmatch results greater than 85% [72]. Thus, while an in vivo crossmatch may provide helpful information, it usually does not change the actual management of the patient. In vivo compatibility testing is rarely done.

Phagocytosis related assays

An in vitro approach to evaluating the potential clinical impact of a red cell antibody is the monocyte monolayer assay (MMA) [73] that has been used to predict the clinical significance of red cell alloantibodies. Mononuclear cells from normal donors are isolated from whole blood using a density gradient, washed, then suspended in tissue culture medium and incubated at 37°C on slides after which the nonadherent cells (lymphocytes) are removed. A monolayer of 95–100% monocytes is left. In a separate procedure, normal RBCs are sensitized with serum from donors having clinically significant antibodies for 1 hour at 37°C. The sensitized RBCs are then suspended in culture medium and incubated with monolayer. At the end of the incubation, unreacted RBCs are removed by washing with PBS. The monolayer is stained and the number of mononuclear cells with internalized or adhered RBCs is quantified.

A wonderful summary of 20 years of data from the developers and primary users of this technique established that a MMA of less than 5%

indicates that incompatible blood can be given without risk of a hemolytic transfusion reaction, although the long-term survival of those red cells may be somewhat shortened. The MMA is an excellent alternative for a 1-hour chromium 51 red cell survival to aid in the decision to transfuse RBCs incompatible with alloantibodies [74].

10.8 Hemolytic disease of the newborn

Laboratory investigation

HDN is one of the classic immunohematology diseases and in the 1940s accounted for as much as 10% of the deaths in fetuses and newborns. The pathophysiology was described by Levine and Stetson in the late 1930s [75]. The mother becomes immunized to a red cell antigen that she lacks but that the infant has inherited from the father. Immunization occurs when minute amounts of fetal red cells cross the placenta during pregnancy. Since immunization occurs during the first pregnancy, usually the disease is not manifest until subsequent pregnancies. The most common cause of clinically important HDN was anti Rh(D). Anti-D continues to be the most common antibody (46% in one study) followed by anti-Kell (15%), and also a combination of antibodies occurs in about one-fourth of patients. The anti-D titer closely parallels the clinical importance of the antibody and it appears that combinations of antibodies were more harmful than single antibody specificities [75a]. HDN due to ABO incompatibility is more common but clinically less severe due to the incomplete development of ABO antigens at birth and the fact that most anti-A or B is IgM that does not cross the placenta. The severe intrauterine hemolysis of HDN causes anemia resulting in congestive heart failure or even death in utero. During gestation, the mother can metabolize the hemoglobin breakdown products but, after birth, hyperbilirubinemia develops and may cause brain damage (kernicterus). As the pathophysiology of HDN was understood, techniques to diagnose it and predict the severity were developed and treatment with exchange transfusion was introduced. Subsequently, intrauterine transfusion was developed to sustain the fetus until it could be delivered and given an exchange transfusion. Modern medicine has added a new twist to the genesis of HDN. In in vitro fertilization, if the egg is obtained from a surrogate donor who is Rh positive, HDN may develop unexpectedly or more severely than expected [76]. HDN is now a preventable disease (see below).

Prior to approximately 1961, the red cell antibody titer and the history of the severity of affected infants in previous pregnancies were the only methods of predicting the severity of HDN in a present pregnancy. This situation was greatly improved by the development of a method for spectrophotometric analysis of amniotic fluid by Liley in 1961 [77, 78]. In this method, the amount of deviation in adsorbance of light at 450 nanometers from a line connecting the adsorbance at 350–700 nm is measured. This difference in adsorbance (ΔOD) measurement is then plotted on a chart based on the gestation stage of the pregnancy, and the

position on the chart is used to predict the severity of HDN. This proved to be an extremely accurate predictor of the severity of the disease.

Monitoring the at-risk fetus

Doppler measurement of peak systolic velocity in the middle cerebral artery (MCA) is increasingly common [79–81]. Peak systolic velocity increases when the anemic fetus must direct more blood to the brain to maintain oxygen levels. This test has been shown to be the most useful for determining HDN. However, after 35 weeks, amniocentesis may be indicated because of a high false-positive rate [79,81].

   Percutaneous umbilical cord blood sampling (PUBS) may also be used as an adjunct to Doppler measurement of MCA peak systolic velocity. In PUBS, a needle, guided by sonography, is used to puncture the umbilical vein near the placenta. The fetal blood is analyzed for hemoglobin levels, hematocrit, blood gases, pH, and bilirubin levels [82]. Some sources recommend that PUBS be used in conjunction with Doppler measurement before physicians are comfortable interpreting Doppler results; however, other sources recommend that PUBS be used sparingly because of increased likelihood of fetal-to-maternal hemorrhage [79].

   About 2 years after Liley introduced the technique for measuring amniotic fluid spectrophotometrically and predicting the severity of HDN, he reported a technique to transfuse severely affected infants [83]. The ultrasound visualization is used to guide a needle into the fetal peritoneal cavity, where the blood is transfused. This intraperitoneal transfusion procedure carried a general mortality of about 7% but greatly improved the prognosis for the most severely affected fetuses. More recently, with the introduction of fetal blood sampling techniques, it has become possible to provide direct intravascular transfusions to the fetus [84].

   The recommended approach to the diagnosis of HDN is briefly outlined in Table 10.8. After determining that the mother is Rh negative, the next step is paternal genotyping. If the father is homozygous for the D-positive trait, no fetal genotyping is indicated because the fetus must necessarily be D-positive. If the father is heterozygous or if paternal genotyping is not possible, fetal genotyping is pursued. In most centers, fetal genotyping is carried out by PCR amplification of cells attained from amniocentesis. Increasingly noninvasive techniques for detection of Rh status in the fetus, such as testing of free fetal DNA in maternal serum, are being

Table 10.8 Steps in the evaluation of potential HDN.

Type mother to be sure she is Rh negative
Type father to determine if he is Rh positive
Carry out antibody screening
If the antibody screening test is positive, identify the antibody
Titer the antibody
Repeat the titer at 16–18 weeks' gestation
If the titer rises or reaches a level beyond the acceptable limit, amniocentesis is indicated

used [85]. If the mother is already immunized, the titer is determined as a baseline for comparison of future samples. Another titer is obtained at about 16–18 weeks, because no treatment will be initiated before then. The subsequent titer is compared with the initial titer. Each laboratory establishes its own guidelines for significance of change in titers. Often, an increase of two dilutions or a value of 1 : 16 are used, but these must be determined locally. If the titer changes more than that locally acceptable, then MCA systolic velocity, amniocentesis, or fetal blood sampling are performed.

Prevention of HDN—Rh immune globulin

From general immunology it has been known that if antibody is given passively along with antigen, immunization can be prevented. Thus in the mid 1960s, experiments began in New York and Liverpool, England, giving Rh-negative male volunteers Rh-positive red cells along with plasma containing anti Rh(D). Immunization was prevented [86, 87]. Immune globulin prepared from plasma containing anti-Rh was then used successfully to prevent immunization in pregnant women who delivered Rh-positive infants [86–88]. The immunoglobulin product is known as Rh immune globulin (RhIG). Although these landmark studies were done about 50 years ago, the mechanism of action of RhIG is still not established.

RhIG must be given within 72 hours of delivery, not for scientific/immunologic reasons but because the clinical trials used that time limit. The present dose of RhIG is 300 micrograms. This will prevent immunization from about 30 mL of whole blood [87, 88]. This dose–response relationship can be used to determine the dose of RhIG to be used in situations of massive fetal maternal hemorrhage or transfusion of Rh positive red cells to an Rh-negative female [89]. If a woman has mistakenly missed receiving RhIG, it should be given as soon as possible but can be attempted up to 28 days after delivery [90].

In a small percentage of women, there was evidence of immunization during pregnancy and so RhIG is now given during pregnancy as well as at delivery [91–93], although the dose is smaller than at delivery. Some failures of RhIG prophylaxis occur because of failure to give RhIG or give the proper dose, or give it at the proper time after abortion, amniocentesis, or massive fetal maternal hemorrhage.

Reactions to RhIG are minor, since it is the same as ordinary immune globulin except that it is produced from plasma with high titer anti-Rh(D).

10.9 Platelet compatibility

Selection of ABO and Rh type for platelet transfusion

ABO antigens are adsorbed onto the surface of the platelet, and the recovery of A_1 platelets transfused into group O patients is decreased [94]. Thus, it is advisable to transfuse ABO-compatible platelets [95] (see Chapter 11). If ABO-compatible platelets are not available, ABO-incompatible platelets should be used rather than withholding platelet transfusion.

Incompatibility between the platelet donor plasma and recipient red cells usually may also be clinically important. If large numbers of platelet concentrates are being transfused to an adult, or if the patient is a small child, incompatible donor plasma may cause a positive DAT and red cell hemolysis (e.g., type O plasma contains anti-A and may react with a type A recipient's red cells). When ABO-compatible platelets are unavailable, consideration may be given to removing some plasma from the platelet concentrate before transfusion. Red cell crossmatching is not necessary prior to platelet transfusion because the platelet concentrate usually contains about 0.5 mL of red cells.

Rh antigens are not found on platelets. However, patients may become immunized to Rh antigens from the red cells contaminating the platelet concentrate. The risk of forming anti-Rh(D) is low and discussed in more detail in Chapter 11). Because of the life-threatening nature of most cases of thrombocytopenia, platelets from Rh-positive donors can be administered to Rh-negative recipients. However, Rh-negative women of childbearing age with a nonmalignant disease should not receive platelet concentrates from Rh-positive donors because of the effect of possible anti-Rh on future pregnancy. If Rh-positive platelets are given to such a patient, Rh immunization can be prevented by the administration of RhIG.

Rh-positive platelets have a normal survival in recipients with anti-Rh(D) [96]; thus, the only concern is a possible transfusion reaction to Rh-positive red cells contained in the platelet concentrate. However, the red blood cell content of platelet products is so small that a significant red cell hemolytic reaction would not be expected, even in a recipient with a preformed anti-Rh(D) antibody.

If an Rh-negative patient receives Rh-positive platelets, it may be desirable to attempt to prevent immunization. This can be done by giving the patient RhIG. Since one standard dose (1 mL) of RhIG will prevent immunization from up to 15 mL of Rh-positive red cells [89,90], this should cover about 25 units of platelets (25 units $\times$ 0.5 mL $=$ 12.5 mL red cells).

Red cell compatibility testing for platelet transfusion

Compatibility testing is not usually done for platelet transfusions because of the small red cell content of the platelet concentrate. However, platelets also contain human leukocyte antigen (HLA) antigens and platelet-specific antigens. Some patients may develop antibodies to these antigens following transfusion, pregnancy, or organ transplantation. When this occurs, transfused platelets have a decreased recovery and a shortened intravascular survival. Therefore, these transfused platelets are less effective in controlling hemorrhage. The management of these patients is discussed in Chapter 11.

10.10 Granulocyte compatibility

Compatibility testing for granulocyte transfusion

Each granulocyte concentrate contains approximately 20 mL of red cells, and thus red cell compatibility testing must be done (see also Chapter 11).

HLA and granulocyte antibodies can interfere with the ability of transfused granulocytes to circulate and localize at sites of inflammation [97]. This problem could be overcome by carrying out leukocyte compatibility testing. However, technical problems make it impractical to do a leukocyte crossmatch of the patient's serum and donor's cells on the day of transfusion. Donors are usually selected by periodically screening the patient's serum against multiple granulocyte donors. Lack of an effective method of ensuring granulocyte compatibility is a major factor in the limited effectiveness of granulocyte transfusion.

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11 Clinical Uses of Blood Components

11.1 Blood component therapy

The development of the closed plastic container system made it possible to separate blood into several of its components (see Chapters 1 and 5). As component production techniques became available, it also became apparent that transfusion therapy was much more complicated than replacing the volume of shed blood with an equal volume of bank blood. Therefore, in developed countries, whole blood is rarely used. Instead blood is separated into its components and these are used for specific situations.

There are a wide variety of patient situations in which optimum transfusion medicine involves replacing only some components of the blood and, in some situations, replacing these components in ratios not ordinarily found in whole blood. This is the basis of blood component therapy, in which specific parts or components of the blood are isolated, stored under conditions optimum for that component, and transfused to patients who need that specific component. About one-third of red cell units are used in surgery, one-third in hematology/oncology, and one-third in other medical/nonsurgical situations. Most transfusions are given in situations of some urgency and only about 10% for nonurgent medical conditions [1].

11.2 Transfusion of components containing red blood cells

Physiology in red cell transfusion decisions

In the early 1900s, it was observed that surgical patients with hemoglobin levels less than 10 g/dL did poorly. Mayo Clinic [2, 3] surgeons who were studying anemia and oxygen transport recommended that a hemoglobin level of 8–10 g/dL be achieved prior to surgery. Although this original recommendation involved a range, the 10 g/dL became accepted in surgical practice and remained so for many years. As advances were made in the understanding of oxygen delivery (D_{O2}) and oxygen consumption (V_{O2}) and as patient management techniques improved, it became clear that patients could survive with much lower hemoglobin concentrations. Thus,

Table 11.1 Physiologic responses to anemia.

Increased cardiac output
Decreased peripheral vascular resistance
Increased red blood cell release of oxygen
Increased heart rate, stroke volume, and contractility
Decreased blood viscosity

Source: Carson JL. Morbidity risk assessment in the
surgically anemic patient. Am J Surg 1995; 170: 32S–36S.

the indications for transfusion began to change. In considering these
changes, it is helpful to briefly review the physiology of oxygenation of
tissues and organs.

In response to anemia, several compensatory mechanisms increase
oxygen delivery (Table 11.1). These include a rightward shift of the
oxyhemoglobin dissociation curve when the hemoglobin falls below 9.9
g/dL [4]; decreased peripheral vascular resistance due to the decrease in
blood viscosity; and increased heart rate, cardiac stroke volume, and
contractility, resulting in increased cardiac output when the hemoglobin
falls below 8–10 g/dL [5–7]. Coronary artery disease, congestive heart
failure, pulmonary disease, peripheral vascular disease, and medications
that affect the heart's ability to increase cardiac output (e.g., beta blockers)
reduce the body's ability to compensate for anemia. Nitric oxide may also
have a role in tissue oxygenation [8]. Hemoglobin transports nitric oxide,
releasing it in areas of hypoxia where the nitric oxide causes vasodilatation
[9], thus improving tissue oxygenation. Thus, the physiology of
tissue/organ oxygenation is so complex that it is unlikely that a single
laboratory value can be used to make red cell transfusion decisions [10].
Several measures have been proposed as indications for transfusion,
including the hemoglobin level, the oxygen extraction ratio, the mixed
venous partial pressure of oxygen ($PV\text{-}_{O2}$), and mixed venous oxygen
saturation ($SV\text{-}_{O2}$). Indications for transfusion could also be based on both
clinical and physiologic factors, including tachycardia, hypotension,
oliguria, $SV\text{-}_{O2} < 60\%$, and $PV\text{-}_{O2} < 30$ mm of mercury [7]. A National
Institutes of Health (NIH) consensus conference concluded that in
addition to the hemoglobin, the patient's age, surgical procedure,
diagnosis, presence of complicating factors such as those described above,
anticipated volume of blood loss, and the cause of the anemia should be
considered [10, 11]. Most patients with hemoglobin concentrations of 10
g/dL or greater do not require transfusion, and most patients with a
hemoglobin level less than 7 g/dL will benefit from transfusion [11, 12].
Another set of suggested indications for red cell transfusion is shown in
Table 11.2 [13]. In normal research subjects, isovolemic reduction of the
hemoglobin to 5 g/dL does not result in inadequate critical oxygen delivery
[14], although energy levels are reduced [15].

Red cells should not be transfused simply to increase the hemoglobin
concentration unless there is a clinically defined need to improve oxygen

Table 11.2 Proposed clinical indicators for red cell transfusion.

6 g/dL	Patients with well-compensated chronic anemia; healthy (American Society of Anesthesiologists [ASA] Class I and some Class II) patients undergoing intentional hemodilution; and patients undergoing hypothermic cardiopulmonary bypass
8 g/dL	Most postoperative coronary artery bypass graft patients, but not those with left ventricular hypertrophy, incomplete coronary revascularization, low cardiac output, poorly controlled tachycardia, or sustained fever
10 g/dL	Patients whose cardiac output is unlikely to increase, with symptomatic cerebrovascular disease, or over age 65

Source: Robertie PG, Gravlee GP. Safe limits of isovolemic hemodilution and recommendations for erythrocyte transfusion. Int Anesthesiol Clin 1990; 28:197–204.

availability. The management of acute blood loss is discussed in Chapter 12, but the most important principle is that blood volume should be restored first using volume expanders, not red cells. The indications of the need for red cell transfusion to provide increased oxygen availability are a combination of clinical and physiologic indicators as described above.

The red blood cell transfusion trigger

The widely used but grammatically incorrect term, transfusion trigger, refers to the hematologic value at which a transfusion is given. Several clinical trials have randomized patients to a liberal or restrictive pretransfusion hemoglobin value to attempt to identify the RBC transfusion trigger [16]. The patients and the definition of liberal or restrictive were all varied and so it is difficult to generalize from these studies. Restrictive ranged from 7 to 9 g/dL and liberal 9, 10 g/dL or 32% or 40%. Many studies were small and endpoints varied from just hemoglobin to some clinical assessment. Clinical settings included cardiovascular or prostate surgery, orthopedics, intensive care, or myocardial infarction. The Transfusion Requirements in Critical Care (TRICC) is one of the best studies. The triggers were 7 g/dL (maintained between 7 and 9 g/dL) versus 10 g/dL (maintained between 10 and 12 g/dL) [17]. As expected, the restrictive group had lower hemoglobin, received fewer transfusions, but also a slightly lower, although nonstatistically significant, mortality. In children in intensive care, a 7 g/dL versus 9.5 g/dL trigger resulted in less blood use with no deleterious effects [18]. From their studies, it appears that increasing the hemoglobin to 10 g/dL improves exercise tolerance. However, the aggregate data are the basis for recommending transfusion in stable patients when the hemoglobin is 8 or even 7 g/dL. However, many clinical factors affect this and the available data is not adequate for firm conclusions regarding transfusing patients, for instance with myocardial or cerebral ischemia, undergoing elective surgery.

Bloodless medicine

Bloodless medicine refers to planned, structured programs using a combination of strategies to avoid red cell transfusion [19–21]. Bloodless

Table 11.3 Aspects of bloodless medicine program.

Preoperatively
   Increase hemoglobin—iron; erythropoietin
   Blood conservation—minimize blood samples for testing
   Autologous blood donation
Intraoperative
   Surgical technique—meticulous hemostasis
   Devices—electrocautery, dissecting media, ultrasound, thermal, coagulation, microwave
   Fibrin sealant
   Patient positioning
   Staged complex procedures
   Anesthesia techniques
   Acute normovolemic hemodilution
   Blood salvage
Postoperative
   Close monitoring for bleeding
   Adequate oxygenation
   Restricted phlebotomy for testing
   Blood salvage
   Pharmacologic agents for hemostasis
   Avoidance of hypertension
   Tolerance of normovolemic anemia
   Careful management of anticoagulants and antiplatelet drugs

Source: Adapted from Goodnough LT, Shander A, Spence R. Bloodless medicine: clinical care without allogeneic blood transfusion. Transfusion 2003; 43:668–676. Spahn DR, Casutt M. Eliminating blood transfusions: new aspects and perspectives. Anesthesiology 2000; 93:242–255. Shander A, Goodnough LT. Objectives and limitations of bloodless medical care. Curr Opin Hematol 2006; 13:462–470. Helm RE, Rosengart TK, Gomez M, et al. Comprehensive multimodality blood conservation: 100 consecutive CABG operations without transfusion. Ann Thorac Surg 1998; 65:125–136.

medicine programs may be helpful in dealing with Jehovah's Witness patients; those with massive transfusion, trauma, autoimmune hemolytic anemia, or multiple red cell antibodies; in situations when red cells are not available; or for patients who prefer to avoid transfusion. Using this multidisciplinary approach involving combinations of drugs, devices, and surgical and medical techniques can eliminate blood transfusion [22]. For patients undergoing elective surgery, steps can be taken preoperatively (Table 11.3) to maintain the hemoglobin or obtaining autologous blood. Intraoperatively, blood salvage techniques are used; and postoperatively, conservative management of anemia can minimize the need for transfusion.

In one example of a comprehensive blood conservation algorithm, the mean rate of allogeneic transfusion was reduced to 16.5% in patients undergoing total hip arthroplasty compared to a 26.1% rate in controls [23]. When the province of Ontario, Canada, assigned a transfusion coordinator to enhance transfusion practice outside the blood bank [24], there was a 14–23% decrease in patients receiving a transfusion and patients who were transfused received less blood. This change was

accomplished by education, preoperative autologous donation, erythropoietin, and intraoperative blood salvage.

Clinical uses of red cells

The most common reason for transfusion is replacement of red cells for oxygen-carrying capacity. The need for increased oxygen delivery may arise due to acute blood loss or chronic anemia. Because of the heterogeneity of patients and clinical situations, there is no single standard indication for red cell transfusion. In deciding whether a patient requires red cell transfusion, the clinical condition of the patient is of primary importance; patients should not be transfused based only on their hemoglobin level.

Restoration of blood volume in acute blood loss

When there is sudden acute blood loss, the major threat to the patient is the loss of intravascular volume and resultant cardiovascular collapse. The amount of blood loss that can be tolerated without replacement depends on the condition of the patient (see Chapter 12). An otherwise healthy individual can tolerate the loss of up to half of the red cell mass without need for replacement [25]. The few studies that deal with the physiology of blood loss and the indications for red cell transfusion have been done in normal animals or essentially healthy humans. In most "normal" patients, the loss of approximately 1000 mL of blood can be replaced by colloid or crystalloid solutions alone [26–28]. However, the indications for transfusion in patients with cardiac disease, coronary atherosclerosis, or other vascular insufficiency are not known. Because many patients have some degree of cardiovascular compromise, they will require red cell replacement after smaller volumes of blood loss. The decision to transfuse red cells must take into consideration the patient's overall condition. If blood loss is judged sufficient to require transfusion, it is not necessary to wait until symptoms such as pallor, diaphoresis, tachycardia, or hypotension develop. Transfusion is with the standard red blood cell component from the stock supply of the blood bank.

Improvement of oxygen-carrying capacity

When anemia has developed over a long period of time, the patient adjusts to lower hemoglobin levels and may not require transfusion despite a very low hemoglobin level. There is no evidence that it is necessary to transfuse a patient to a "normal" hemoglobin prior to surgery [11], nor is there any specific hemoglobin value above which patients feel better or have better wound healing. In patients with chronic anemia, transfusion should be used only as a last resort, since it may suppress erythropoiesis.

Transfusion in preparation for surgery

There are no scientific or well-described clinical data to support the practice of many anesthesiologists and surgeons to transfuse patients when the hemoglobin is 10 g/dL or less. It appears that many patients would not

be at risk if transfusion was withheld until the hemoglobin was less than 7 g/dL [11], and in normal human research subjects a hemoglobin of 5 g/dL does not result in inadequate tissue oxygenation [14] but does decrease exercise tolerance [15]. In baboons subjected to normovolemic anemia (exchange transfusion using dextran for replacement of red cells), death occurs at hematocrit levels of about 5% [11, 29, 30]. This decline in hemoglobin was accompanied by a linear decrease in mixed venous oxygen tension, an increase in extraction of oxygen from the red cells, and an increase in cardiac output, allowing oxygen consumption to remain unchanged until the hematocrit fell to 10% or less [11]. The authors concluded that the normal heart has a remarkable capacity to adjust to acute normovolemic anemia and suggested that most patients should not receive a transfusion unless the hemoglobin is 7 g/dL or less. Few patients, especially those with normal cardiac function, would require transfusions at hemoglobin levels of 10 g/dL. Transfusion to patients with hemoglobin between 7 and 10 g/dL should be based on clinical assessment of the particular patient [10, 29, 30].

Uses of specific red blood cell components
Red blood cells
Red cells are the component of choice for any patient with severe anemia. Most patients who require red cell replacement do not also need intravascular volume replacement. In patients who do need both intravascular volume and red cell replacement, crystalloid or colloid solutions, not human plasma, are the preferred solutions for intravascular volume replacement. These latter solutions have few, if any, adverse effects and their use allows the plasma from the original unit of whole blood to be used for the production of coagulation factor concentrate. Thus, almost all transfusions given for red cell replacement are red cells.

Clinical effects of stored red blood cells
The changes that occur during RBC storage include deformability, ATP and 2,3-DPG levels, nitric oxide release, cytokine accumulation, intra and extracellular electrolytes, and others only now being studied with contemporary techniques [31]. Since most blood banks use a first-in-first-out inventory management system, the longest stored red cells are provided to patients most likely to be transfused. Thus, often the sickest patients receive the oldest red cells. Some reports suggest that long stored red cells have adverse clinical effects including increased mortality, post-operative infection, pneumonia, renal or multiorgan failure. Lelubre et al. [32] reviewed clinical trials in cardiovascular surgery, trauma, colorectal surgery, and ICU patients. All of the studies have major shortcomings including: retrospective, small, single center using different endpoints and different ways of defining the age of all transfusions [32]. The potential clinical impact of long stored RBCs is an extremely important issue with implications for patients and blood banks. A group of basic science projects has been funded and a major clinical trial (RECESS) is underway, both funded by the NHLBI, but the issue is not resolved.

Table 11.4 Clinical situations involving leukocyte or platelet antibodies.

Antibody	Clinical problem
Leukocytes	Acute graft rejection
	Inability to obtain compatible organ
	Rejection of transplanted marrow
	Febrile nonhemolytic transfusion reaction
	Transfusion-related acute lung injury
	Poor response to granulocyte transfusion
	Refractoriness to platelet transfusion
	Increased postoperative infection[a]
	Increased cancer recurrence[a]
Granulocytes	Alloimmune neonatal neutropenia
	Transfusion-related acute lung injury
	Febrile nonhemolytic transfusion reaction
	Poor response to granulocyte transfusion
	Transfusion-related acute lung injury
Platelets	Refractoriness to platelet transfusion
	Alloimmune neonatal thrombocytopenia

[a]These consequences may be due to immune modulation but this is not established.

Whole blood

In parts of the world, especially developing countries, whole blood is used because the technology and resources for production of blood components are not available. In these settings, use of whole blood is appropriate. Many transfusions are for trauma or peripartum hemorrhage—situations in which whole blood is satisfactory. For transfusion of stable anemic patients in these settings such as malaria or hemoglobinopathies, the plasma can be removed from sedimented red cells at the time of transfusion.

Leukocyte-depleted red cells

Transfusion has several immunologic effects (Chapter 14). These include alterations of immune responsiveness and/or alloimmunization to red cell, platelet, or leukocyte antigens (Table 11.4). These immune consequences can be rather common or sometimes very serious. It appears that most of these immune consequences are due to leukocytes in the donor blood and thus leukodepleted blood components are used to prevent these immunologic consequences (Table 11.5).

Table 11.5 General approach to the use of leukodepleted red cells.

Treatment of patients with multiple febrile transfusion reactions
Prevention of alloimmunization to leukocytes and platelets
Prevention of transmission of viruses such as cytomegalovirus
Prevention of immunomodulatory effects of transfusion

Clinical Uses of Blood Components

Table 11.6 Clinical situations in which leukodepleted red cells are recommended.

Bone marrow or peripheral blood stem cell transplantation
Acute leukemia
Chronic leukemia
Congenital platelet function abnormalities
Congenital immune deficiency syndrome
Hematologic malignancies potentially treated with stem cell transplantation
Solid tumors potentially treated with stem cell transplantation
Intrauterine transfusions
Exchange transfusion for hemolytic disease of the newborn (HDN)
Hemoglobinopathy or thalassemia
Renal, hepatic, or cardiac failure if potential transplant
Surgical patients[a]
Cancer patients[a]

[a]Optional recommendation due to lack of consensus regarding the immune modulation effect.

The kinds of patients who should receive leukodepleted red cells include any patient with a disease potentially treated by marrow or organ transplantation and those who will receive multiple transfusions during their life such as those with hemoglobinopathies. A specific suggested list of such patients is provided in Table 11.6.

When patients with leukocyte antibodies receive blood containing incompatible leukocytes, febrile transfusion reactions may occur (see Chapter 14). These leukocyte reactions do not cause red blood cell hemolysis but can be extremely uncomfortable for the patient and are potentially fatal. The frequency and severity of leukocyte transfusion reactions are directly related to the number of incompatible leukocytes transfused [33, 34]. Therefore, leukocyte-depleted red cells are indicated for patients who have repeated febrile transfusions reactions.

Leukodepleted red cells are effective in preventing alloimmunization and refractoriness to platelet transfusion [35–40]. The use of leukodepleted components is the optimum therapy for patients who may receive many transfusions and in whom platelet refractoriness should be avoided.

Preventing cytomegalovirus (CMV) transmission by the removal of the leukocytes where the virus is thought to reside [41] is an ideal strategy because of the difficulty in finding an adequate supply of CMV antibody-negative blood components. Leukodepletion reduces the circulating viral load [41], and studies comparing components leukocyte-depleted by filtration with CMV antibody-negative components established that filtered leukocyte-depleted components are effective in preventing CMV transmission [42–46]. There has been some debate about whether the pivotal study [46] provides adequate data on which to base the practice of using leukodepleted blood components for prevention of CMV. In the filtration arm, three of 250 patients demonstrated CMV seroconversion, and in the antibody-screened arm, two of 252 demonstrated CMV seroconversion. This difference was not significant.

However, of the three patients receiving filtered components, all developed CMV disease, whereas of the two patients receiving CMV antibody-negative components, none developed CMV disease but only seroconversion. These differences are not statistically significant. A more recent study [47] renews the concern about the value of leukodepleted red cells for CMV prevention. In that study, 4% of patients who received leukodepleted red cells developed CMV seroconversion compared with 1.7% of those receiving CMV antibody negative red cells ($p < 0.05$). Despite this study, with the increasing use of leukodepleted red cells, it has become the practice in many institutions to use components leukodepleted by filtration interchangeably with CMV antibody-negative components for prevention of transfusion-transmitted CMV [48].

Leukocyte antibodies interfere with finding a compatible organ for transplantation [49], and so leukodepleted red cells are recommended for patients such as those with end stage renal disease that are potential transplant recipients. Leukocyte antibodies can also cause rejection of transplanted hematopoietic stem cells [50], and this along with the need to prevent platelet refractoriness makes leukodepleted red cells indicated for patients with hematologic malignancies or those who may need a hematopoietic cell transplant (Table 11.6).

Because there is no in vitro test to establish a patient's susceptibility for graft versus host disease (GVHD), nor is there a known threshold of leukocyte dose that will cause GVHD, leukocyte depletion is not an acceptable method for preventing transfusion-associated GVHD.

Although some filters may remove some bacteria from units of whole blood [51–56], filtration and leukodepletion are not satisfactory methods for removal of bacteria from contaminated units.

It has been known since the 1970s that patients who received red cell transfusions experienced better renal graft survival than nontransfused patients [57]. This indicated that transfusion was associated with an immunomodulatory effect. Many studies during the past decade have suggested that this transfusion-related immunomodulatory (TRIM) effect may influence the rate of postoperative infection and the likelihood of cancer recurrence. A large number of studies have not resolved this issue [58–68] (see Chapter 14; TRIM). In animals and humans there is reason to believe that the immunomodulatory effect is due to the leukocytes contained in the transfusion components. Thus, interest has focused on the potential value of leukocyte-depleted components in preventing the immunomodulatory effect. It is clear that transfusion does cause an immune effect, but the data are not adequate to settle the role of leukodepleted red cells in proventing this immune modulation [69].

The accepted indications for leukodepleted red cells involve a substantial portion of patients receiving red cell transfusion—well over 50% in some hospitals. Because of the difficulty maintaining two inventories and the belief that the immune effect on postoperative infection and cancer occurrence might be real, some hospitals and some countries have converted to 100% leukodepleted red cells. In Canada, this has resulted in decreased mortality in high-risk patients [70] and in premature infants

Table 11.7 Indications and nonindications for leukocyte-depleted blood components.[a]

Established indications	Prevention of recurrent nonhemolytic febrile transfusion reactions to red blood cell transfusions
	Prevention or delay of alloimmunization to leukocyte antigens in selected patients who are candidates for transfusion on a long-term basis
Nonindications	Prevention of transfusion-associated graft versus host disease
	Prevention of transfusion-related acute lung injury due to the passive administration of antileukocyte antibody
	Prevention of transfusion-related acute lung injury due to the passive antibody
	Patients who are expected to have only limited transfusion exposure
	Acellular blood components (for example, fresh frozen plasma, cryoprecipitate)
Unresolved	Surgical patients
	Cancer patients

Source: Modified from Lane TA, Anderson KC, Goodnough LT, et al. Leukocyte reduction in blood component therapy. Ann Intern Med 1992; 117:151–162.
[a]Unless otherwise stated in the text, a third-generation filter or an equivalent technique should be used to reduce the leukocyte content of blood components.

[71]. I recommend routine use of leukodepleted blood components for all patients to avoid the known complications and potential immunologic effects (Table 11.6). Some have opposed this wider use of leukodepleted red cells because of the additional cost, but the reduced complications following use of leukodepleted red cells may make this no more costly than present practice [72]. A summary of thinking about the use of leukodepleted red cells is shown in Table 11.7.

Washed red cells
Washed red cells are composed of red cells suspended in an electrolyte solution; most plasma, platelets, and leukocytes have been removed. After washing, the storage period is 24 hours because the washing has been carried out in an open system. Thus, washed cells are used only for specific indications and are not practical for the general inventory. Because the major advantage is the removal of the plasma by washing, washed red cells are indicated for patients who have reactions caused by plasma such as patients with IgA deficiency that can have severe, sometimes fatal, anaphylactic reactions when exposed to plasma containing IgA (see Chapter 14). Washed red cells are also used for patients who have hives, urticaria, or allergic reactions to plasma that may be due to cytokines or histamine (see Chapter 14). Although washed red cells have been used to prevent febrile reactions due to transfused leukocytes, the availability of high-efficiency filters for leukocyte removal eliminates this indication by providing another blood component with more extensive leukocyte depletion.

Frozen deglycerolized red blood cells

The use of frozen red cells is for long-term storage of red cells from donors with very rare antigen phenotypes. This usually means donors who lack a very high-frequency antigen or in whom the combined absence of several antigens makes the red cells extremely rare. These individuals may donate for general allogeneic use or for their own use. Although its use is much more limited than was anticipated by advocates of frozen red cells during the 1970s, this is an extremely valuable strategy for blood banks and transfusion medicine. Substantial stockpiles of very rare red cells can be established. Individual blood banks may maintain some rare red cells, but stockpiles of rare red cells are available through the American Association of Blood Banks (AABBs) and the American Red Cross Rare Donor Registries. These rare donor registries can be accessed through any AABB-accredited immunohematology reference laboratory or American Red Cross regional blood center. At one time it was thought that if the instruments, costs, and technology could be simplified, depots of frozen red cells could be used to supplement the general blood supply in times of shortage. The technology was never simplified and it has not become a practical way to provide blood for the general inventory in times of shortage.

In the past, frozen red cells were used as a leukocyte-poor red cell component instead of centrifuged, filtered, or washed red cells. However, these uses are no longer recommended because of the availability of high-efficiency filters that provide a more leukodepleted component at lower cost and without a reduced storage period. There is no advantage of frozen red cells over washed red cells for patients who have allergic reactions to plasma or anaphylactic reactions in IgA-deficient patients because both components undergo a washing step that removes virtually all of the plasma.

Because of the extensive washing, there was speculation that frozen deglycerolized red cells may have no risk of transmitting viruses such as hepatitis [73, 74], but other subsequent studies established that frozen deglycerolized red cells can transmit hepatitis [75, 76], human immunodeficiency virus (HIV), or CMV. One study [77] indicates that frozen deglycerolized red cells may prevent transmission of CMV. However, more extensive and convincing data are available regarding the effectiveness of red cells leukodepleted by filtration in preventing CMV transmission and there is no reason to use frozen deglycerolized red cells for this purpose.

Effects of red blood cell transfusion
Circulation

When a unit of red cells or whole blood is transfused rapidly (within 30–60 minutes) to a patient with a normal blood volume, the blood volume is increased by the volume of the component. If the volume of blood transfused is very large or the patient has compromised cardiovascular function, the central venous pressure may increase. In most patients the intravascular volume remains about 10% above pretransfusion levels after

24 and even 48 hours [78]. Thus, patients who receive large volumes of blood or in whom cardiovascular function is compromised must be monitored for the effects of transfusion. If plasma alone is transfused, the blood volume may readjust more rapidly, returning to normal in 24 hours and often in only a few hours [79]. Some patients, such as those with chronic renal disease, may require prolonged periods to readjust their blood volume [78].

Hemoglobin concentration

The effects of red blood cell transfusion on the recipient's hemoglobin concentration and hematocrit will be affected by the recipient's blood volume, pretransfusion hemoglobin level, clinical condition (stable, bleeding, etc.), and by the hemoglobin content of the donor unit. The rate of shift of water from the intravascular space and reequilibration of the intravascular volume will determine when after transfusion the hemoglobin value will reflect the new equilibrium. Because plasma may take up to 24 hours to exit the intravascular space, the new hemoglobin value may not be established until that time. The projected increase in hemoglobin concentration can be estimated. For instance, a hypothetical patient with a blood volume of 5000 mL and hemoglobin concentration of 8 g/100 mL has a total of 400 g of hemoglobin. If a unit of red cells containing 60 g of hemoglobin (200 mL of red cells with hemoglobin of 30 g/dL and 100 mL of suspension medium) is transfused, the blood volume becomes 5300, the total hemoglobin 460 g, and the hemoglobin concentration 8.7 g/dL. If the patient is able to readjust the blood volume to baseline levels within 24 hours, the result will be a 5000 mL blood volume, 460 g of total hemoglobin, and a hemoglobin concentration of 9.2 g/dL. Thus, a hemoglobin increase of approximately 1 g/dL can be expected from one unit of red cells in an average size adult. Red cell transfusions are not given on a dose per weight basis, except in neonates and children. Thus, the usual dose is one or two units of red cells. Since the hemoglobin content of donor units can vary as much as 50%, Arslan, et al [80] tested a strategy of matching the hemoglobin content of the red cell units to the blood volume of the recipient and the desired elevation in hemoglobin. They observed a 30% reduction in the number of red cells used compared to the original order from the physician and so this approach might improve the rationale use of the hemoglobin content of different red cell units [80].

Red cell production

Following transfusion to stable anemic patients, the hemoglobin returns to pretransfusion on the life span of the transfused red cells [78]. In addition, there is a decrease in the reticulocyte count and erythropoietin levels [78]. Intentional transfusion programs for patients with thalassemia or sickle cell disease result in suppression of red cell production. These observations illustrate that transfusion of red cells results in a decrease in the recipient's red cell production rate, probably due to suppression of erythropoietin production as a result of the increased red cell mass [78]. Thus, patients with a stable chronic anemia may receive less than the expected benefit

from red cell transfusion, or the benefits may not be as long lasting as expected, since the patients' hemoglobin may fall to pretransfusion levels due to diminished production of their own red cells and the clearance of the transfused cells.

Survival of transfused red blood cells

The normal red cell has a life span of approximately 120 days. This is reflected after transfusion in the fact that approximately 1% of the donor cells are lost each day [78]. Each unit of blood contains red cells of all ages between 1 and 120 days. As the unit of blood is stored, the red cells continue to age, and the senescent red cells are removed from the circulation within 24 hours after transfusion. Thus, when stored blood is transfused, there is a slight decrease in the proportion of red cells surviving 24 hours after transfusion depending on the length of time the blood has been stored. For instance, approximately 80% of red cells stored in the anticoagulant AS-1 (Adsol) for 42 days survive following transfusion. These red cells then survive normally and are destroyed linearly with a mean half-life of 50–60 days [78]. The survival of transfused red cells is also affected by the recipient's health and may be decreased in patients with active bleeding (and iatrogenic blood loss), hemolytic anemia due to defects extrinsic to the red cells (autoantibodies, hypersplenism), and chronic renal or liver failure [78].

Immune system

Red cell transfusion has immunologic effects including alloimmunization to red cell or leukocyte antigens (if not leukodepleted), and immunomodulation. These issues are discussed more extensively in Chapter 14 and in the section in this chapter on the use of leukodepleted red cells.

11.3 Transfusion of components and derivatives containing coagulation factors

Therapeutic agents containing coagulation factors can be prepared either from units of whole blood or from plasma by large-scale fractionation (see Chapter 5). The whole blood-derived components that can be used to replace coagulation factors are fresh frozen plasma (FFP), plasma, and cryoprecipitate. Plasma-derivative products are factor VIII, factor IX, antithrombin III (AT III), and fibrinogen concentrates. Fibrin sealant or glue can be prepared either from units of whole blood or on a large scale from pools of plasma.

Fresh frozen plasma

FFP is used for documented single coagulation factor deficiencies, multiple factor deficiencies, warfarin reversal, and massive transfusion [81, 82] (Table 11.8). FFP can be used for the treatment of deficiencies of factors II, V, VII, IX, X, or XI because specific component therapy is usually not

Table 11.8 Indications for the use of fresh frozen plasma.

Replacement of isolated coagulation factor deficiencies
Reversal of warfarin effect
Massive blood transfusion
Antithrombin III deficiency
Immunodeficiency
Thrombotic thrombocytopenic purpura

Source: NIH Consensus Conference. Fresh frozen
plasma: indications and risks. JAMA 1985;
253:551–553.

available for these factors. FFP can also be used to reverse the warfarin
effect if this must be done more quickly than could be accomplished by the
administration of vitamin K. In addition, FFP can be used to treat
deficiencies of protein C and protein S. FFP may be helpful in patients
experiencing massive transfusion (see also Chapter 12). FFP can be used as
a source of antithrombin III, although concentrates of antithrombin III are
now available. FFP is also useful in patients with secondary
immunodeficiency associated with severe protein-losing enteropathy,
although parenteral nutrition is often effective in preventing this
immunodeficiency. FFP is also used regularly as replacement solution for
plasma exchange in patients with thrombotic thrombocytopenic purpura
(TTP). FFP is not indicated for use as a volume expander or a nutritional
source [82]. FFP is often used to correct slightly elevated international
normalized ratio (INR) in preparation for invasive diagnostic procedures
but there is no evidence that this is beneficial [83].

24-hour plasma (FP24)
This is plasma frozen more than 8 and less than 24 hours after collection. It
contains normal amounts of Factor V but only an average of about
55–75% Factor VIII [84]. FP24 is often used interchangeably with FFP.

Plasma
There are few indications for the use of plasma. Because it was not
separated from whole blood and frozen within eight hours of collection,
plasma is deficient in factors V and VIII. It may be used to treat deficiencies
of coagulation factors other than V and VIII if concentrates of those factors
are not available. Examples are factors II, V, VII, and XI. Plasma could also
be used instead of FFP for replacement therapy in liver disease or for
reversal of warfarin effect. Some transfusion medicine physicians believe
that plasma can be used in patients with disseminated intravascular
coagulation (DIC) or hemodilution due to massive transfusion. The need
for factors V and VIII make FFP preferable. Cryoprecipitate-poor plasma
has been used as replacement solution when TTP is treated with plasma
exchange (see Chapter 19). This plasma may also be provided to
manufacturers for production of albumin, immunoglobulins, or
laboratory reagents.

Solvent–detergent plasma

Treatment of fresh plasma with a combination of solvent tri-n-butyl-phosphate and the detergent Triton X100 inactivates lipid envelope viruses while retaining most coagulation factor activity. The process must be done on a large scale, and plasma from about 2500 donors is pooled for the solvent–detergent (SD) process. The product has little, if any, risk of transmitting lipid envelope viruses such as HIV, hepatitis C virus (HCV), and hepatitis B virus (HBV) but can transmit nonlipid envelope viruses such as parvovirus [85]. An SD plasma product was available in the United States, but reports of thrombosis in TTP patients undergoing plasma exchange with SD plasma [86] and deaths in patients receiving SD plasma while undergoing liver transplantation [87] led to withdrawal of SD plasma from the market, and it is no longer available. It is postulated that these thrombotic complications were due to decreased protein S and plasmin inhibitor activity in SD plasma [86, 87]. A different SD plasma, Octaplas, which is available in Europe, has higher, although not normal, levels of protein S and plasmin inhibitor [88] and has not been associated with thrombotic events.

Cryoprecipitate

Soon after the development of cryoprecipitate, it became the mainstay of treatment for hemophilia A because it was the first concentrated form of factor VIII [89, 90]. This remained the product of choice until commercial coagulation factor concentrates became available in the mid- to late 1970s. When commercial concentrates of fibrinogen were removed from use because of the high risk of hepatitis [91], cryoprecipitate became used as a source of fibrinogen [92–94]. This has become the major use of cryoprecipitate in multiple situations [94]. The usual dose of cryoprecipitate when it is used as a source of fibrinogen is one bag per 5 kg body weight of the recipient or one bag should increase the fibrinogen level by 5–10 mg/dL [94]. Another use of the fibrinogen in cryoprecipitate is as fibrin sealant or glue. Cryoprecipitate was used as a source of concentrated von Willebrand factor but vWF concentrate is now available and it is used to treat active hemorrhage or to prepare for invasive procedures in patients with severe forms of the disease. Some physicians may not understand the composition of cryoprecipitate, since as much as 24% may be used inappropriately [95].

Fibrin sealant (glue)

Since the early part of this century, various crude forms of fibrinogen have been used to attempt to control localized bleeding [96]. Fibrin sealant or glue refers to the use of fibrinogen in some form along with thrombin as a topical adhesive to control bleeding [97]. For several years, many blood banks dispensed cryoprecipitate in syringes along with thrombin for use by surgeons [98]. More pure forms of fibrinogen are now being produced with chemical additives. The composition and characteristics of these products are listed in Table 11.9. They contain fibrinogen, factor XIII, and human thrombin. They are reconstituted with saline at 37°C within about

Table 11.9 Composition of fibrin sealant.

	Human fibrinogen (mg/mL)	Human factor XII (U/mL)	Human thrombopoietin (U/mL)	Bovine aprotinin (KIU/mL)	Virus-inactivated fibrinogen	Virus-inactivated source thrombin
IMMUNO AG (Austria)	70–115	10–50 (Europe) <1 (USA)	4 and 500 (Europe) 500 (USA)	3000	Two-step vapor heat at 60/80°C	Two-step vapor heat at 60/80°C
Centeon Pharma GmbH (Germany)	65–115	40–80	400–600	900–1100	Wet heat, 10 hours at 60°C	Wet heat, 10 hours at 60°C
LFB-Lille (France)	115	10–30	500	3000	SD [a]	SD
SNBTS (Scotland)	40	10	200	None	Dry heat, 72 hours at 80°C	SD
Haemacure Biotech (Canada)	50–70	20–40	150–250	None	SD, nanofilter dry heat, 1 hour at 100°C	SD, nanofilter dry heat, 1 hour at 100°C
Baxter/American Red Cross (USA)	100	24	300	None	SD	SD, nanofilter
Melville Biologics (USA)	50–95	3–5	200	None[b]	SD, UVC[c]	SD, UVBC

Source: Levitsky S. Further information on the fibrin sealant conference. Transfusion 1996;36:845. Letter to the editor.

[a]Solvent–detergent treatment.

[b]0.1 M epsilon amino caproic acid as excipient.

[c]Ultraviolet C light.

10 minutes [96]. Fibrin sealant may be used for either its hemostatic or its adhesive properties. To achieve hemostasis, surgeons use fibrin sealant (a) to deal with microvascular bleeding in cardiovascular surgery to reduce mediastinal drainage, (b) to seal synthetic vascular grafts, (c) to seal bleeding surfaces of the liver or spleen, (d) in maxillofacial surgery, (e) for sealing dura, and (f) for peripheral nerve repair (Table 11.10).

Table 11.10 Indications for fibrin sealants.

Product	Source	Indications
Artiss	Baxter	Adhesion of autologous skin grafts in burn surgery
BeriPlast P	Behring	Tissue adhesion, suture support, and hemostasis in general surgery
Evicel	Omrix	Adjunct to hemostasis in general surgery
TachoSil	Nycomed	Absorbable patch as adjunct to hemostasis in cardovascular surgery
Tisseel	Baxter	Hemostatsis in cardiopulmonary bypass and splenic surgery
Quixil	Omrix	Adjunct to hemostasis in hepatic and orthopedic surgery

Controlled trials have demonstrated that fibrin sealant significantly reduces the time required to achieve hemostasis in vascular surgery [99], improves hemostasis after operation following cardiac operations [100], and reduces blood loss from vascular cannulation in neonates [96]. The adhesive properties of fibrin sealant are used to promote the union of middle ear bones in otolaryngologic surgery, to enhance skin grafting, to seal bronchopulmonary fistulas, and as a matrix for repair of bone defects [96].

Since fibrin glue is made from human plasma, transmission of diseases might occur. The likelihood of this is not known [97] but seems to be quite low [96]. All of the commercially prepared products involve at least one viral inactivation step, and it is hoped that this will render these products free of viral disease transmission. Fibrin sealant has been associated with the development of antibodies to the bovine thrombin it contains as well to factor V [98, 101, 102]. With the availability of commercial fibrin sealant, the use of cryoprecipitate and locally produced syringe thrombin kits has nearly disappeared. Wound dressings containing a fibrin sealant are also available primarily for military use.

Deficiency of multiple coagulation factors

In considering the use of coagulation components, it is convenient to separate situations requiring replacement of multiple factors from those requiring an isolated component. In all of these situations, coagulation factor levels of only 20–30% are adequate to achieve normal hemostasis (Figure 11.1).

Prothrombin-complex deficiency

The most common combination deficiency of coagulation factors involves those dependent on vitamin K for synthesis. This usually occurs as a result
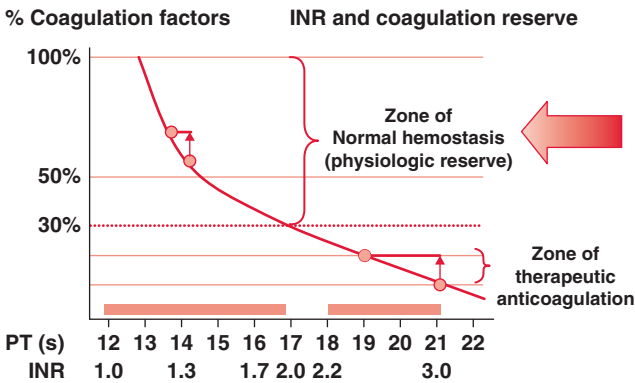
Figure 11.1 Coagulation factor levels in relation to hemostases and coagulation test results. (Reproduced with permission from Callum J, Dzik W. Component therapy before beside procedures. In: Mintz PD, ed. Transfusion Therapy: Clinical Principles and Practices, 3rd ed. AABB Press; Bethesda, MB, 2011, p. 6.)

of liver disease or warfarin therapy. This type of coagulation disorder is best managed by treating the underlying condition with or without vitamin K administration. However, the coagulation factors can be replaced using plasma of any age, since these coagulation factors do not deteriorate during storage of whole blood between 1°C and 6°C (see Chapter 5). For reasons that are not clear, two units of FFP have become the usual dose for reversing the effects of warfarin. This is inadequate [83, 103, 104]. In an ordinary-size adult this restores the prothrombin time to normal in only about 10–12% of patients [103], and six or more units are probably needed. A recent clinical trial of pathogen-inactivated FFP nicely illustrates that the usual dose of two units does not normalize the prothrombin time (Figure 11.2) [104]. There are no clinical trials that establish the clinical value to these small doses of FFP prior to invasive procedures such as liver or kidney biopsy, although this is one of the largest uses of FFP [83, 105]. Commercial concentrates containing factor IX
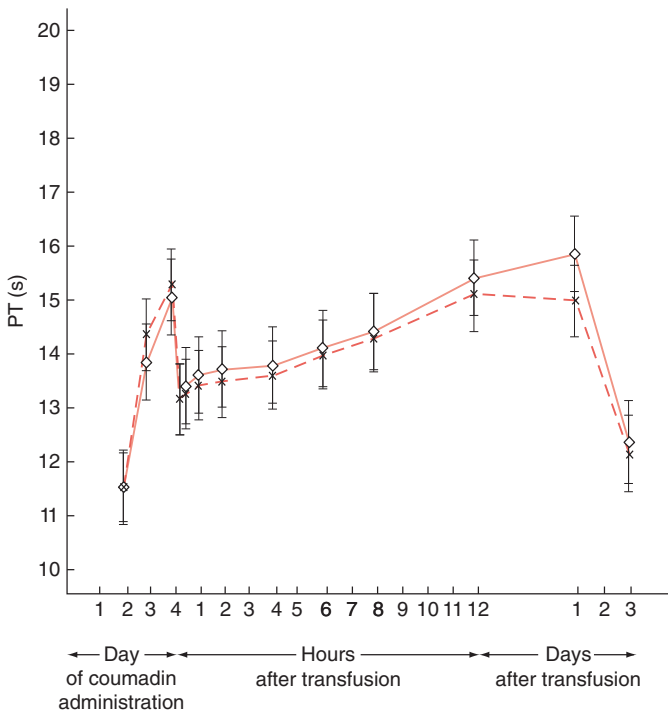
Figure 11.2 The prothrombin time (PT) graphed against time for all study infusions. Warfarin given over 4 days before infusion increased the PT to 15 seconds. Infusion of approximately 12 mL per kg resulted in a 2-second decrease of the mean PT down to 13 seconds for both test FFP and control FFP. (×) Mean control infusion; (◊) mean test infusion; bars represent 95% CIs. (Reproduced from Hambleton J, Wages D, Radu-Radulescu L, et al. Pharmacokinetic study of FFP photochemically treated with amotosalen (S-59) and UV light compared to FFP in healthy volunteers anticoagulated with warfarin. Transfusion 2002; 42:1302–1307.)

(II, VII, IX, X) complex can be used to replace an acquired deficiency of multiple factors caused by warfarin [106].

Massive transfusion

Bleeding due to multiple coagulation factor deficiency can occur in massive transfusion as a result of dilution of coagulation factors. Since virtually all red cell components today contain little plasma, coagulation factors are not replaced. Management of this situation is described more fully in Chapter 12, but if coagulation factor replacement is necessary in these patients, FFP is recommended. Patients, undergoing massive transfusion may also develop DIC due to the severity of the underlying problem. Thus, a bleeding diathesis may develop that is a combination of the dilutional coagulopathy of massive transfusion and the "consumption coagulopathy" of DIC.

Disseminated intravascular coagulation

Treatment of the underlying cause of the DIC is essential; without it, transfusion of blood components merely adds more substrate for the coagulation process. In mild forms of DIC, transfusion is usually not necessary. However, in the more extreme forms there is usually a deficiency of factors V and VIII, fibrinogen, and platelets. Replacement of coagulation factors in the management of DIC should be based on laboratory abnormalities and not on arbitrary formulas. When replacement is necessary, usually FFP is used to replace all factors.

Deficiency of single coagulation factors
Deficiency of factor VIII

It is beyond the scope of this book to describe the management of patients with hemophilia A or congenital factor VIII deficiency. A brief description of the available products containing factor VIII and their use is presented. Factor VIII can be replaced with cryoprecipitate, FFP, or a number of factor VIII concentrates. With its discovery in the 1960s, cryoprecipitate became the mainstay of treatment for hemophilia A. Today, cryoprecipitate is used very little because of the availability of factor VIII concentrates in developed countries, but cryoprecipitate is very satisfactory in less developed countries.

The following approach can be used to determine the dose of different components. One unit of factor VIII equals the factor VIII activity of 1 mL of fresh normal pooled plasma. Factor VIII levels are usually reported as a percentage of normal. The intravascular recovery of factor VIII is close to 100%, so the amount of factor VIII required to achieve a specified factor VIII level can be estimated as follows:

$$\text{Weight (kg)} \times 70\,\text{mL/kg} = \text{blood volume(mL)(adults)}$$
$$\text{Blood volume(mL)} \times (1 - \text{hematocrit}) = \text{plasma volume(mL)}$$
$$\text{Plasma volume(mL)} \times (\text{desired factor VIII level\%} - \text{initial factor VIII \%})$$
$$= \text{units factor VIII required}$$

Example: To raise the factor VIII level to 50% in a 70-kg patient with a hematocrit of 40% and a factor VIII level of 0%:

$70 \, \text{kg} \times 70 \, \text{mL/kg} = 4900 \, \text{mL}$
$4900 \, \text{mL} \times (1 - 0.40) = 2940 \, \text{mL}$
$2940 \, \text{mL} \times 0.5 = 1470 \, \text{units}$

If factor VIII concentrate is to be used, the number of units will be known from the manufacturer's quality control tests or vial labels. Thus, the number of vials needed can be determined. If cryoprecipitate is to be used, each bag of cryoprecipitate contains about 100 units of factor VIII; thus in the above example, 15 bags of cryoprecipitate will be required.

In cases where the initial factor VIII level is not known, it can be assumed to be 0% in a patient with severe classic hemophilia A. Since factor VIII is almost entirely intravascular, it can be assumed that virtually all of the injected factor VIII will be recovered. The half-life of factor VIII after transfusion is 8–12 hours, so it is usually necessary to repeat the factor VIII transfusion at 8- to 12-hour intervals to maintain hemostatic levels. Some hemophiliacs may have an inhibitor that causes a shortened half-life of factor VIII after transfusion. The calculations described above provide an estimate of the factor VIII level attained immediately after transfusion, and the dosage should be adjusted so that the minimum desired level is reached just prior to the next transfusion.

Example: If the desired minimum factor VIII level is 30% and the half-life of factor VIII is 12 hours, it is necessary to elevate the patient's initial factor VIII to 60% so that the level just before the next dose will be 30%. From the example above, the patient's plasma volume is 2940 mL. The units required to elevate factor VIII to 60% are $2940 \, \text{mL} \times (0.60 - 0)$ or 1764 units. Twelve hours later, half of the factor VIII remains, thus the factor VIII level is 30%. For the next dose of factor VIII, $2940 \times (0.60 - 0.30)$ or only 882 units are required to elevate factor VIII to 60%. Once the dose of factor VIII is determined, the amount of cryoprecipitate or factor VIII concentrate necessary can be calculated easily. The duration of treatment with factor VIII depends on the type and location of the hemorrhage and the clinical response of the patient. Depending on the reason for administration of factor VIII, the dose may range from 25 to 50 units/kg, providing blood levels of factor VIII of 30–80%.

Some hemophilia A patients develop factor VIII antibodies. A detailed description of this situation is beyond the scope of this book. In brief, approaches have involved use of porcine factor VIII, plasma exchange, desensitization strategies, rituxamab, or achieving hemostasis by bypassing factor VII. This can be done with activated prothrombin complex or activated factor VII concentrates. Factor VIIa is now approved by the US Food and Drug Administration (FDA) and is very effective for this purpospe [107].

Deficiency of factor IX
Isolated inherited deficiency of factor IX (hemophilia B) is clinically similar to hemophilia A. Factor IX is stable in plasma when stored at 4°C

or at $-20°C$. Thus, stored blood, liquid plasma, or FFP can be used to replace factor IX. However, it is difficult to replace large amounts of factor IX because of the volume of these components. Commercial preparations containing concentrated factor IX are available and are the recommended product for large amounts of factor IX. Factor IX is available in one of three forms: factor IX, factor IX complex (II, VII, IX, X), and activated factor IX complex. The factor IX complex products are known as prothrombin complex concentrates [106, 108]. The approach to use of factor IX is similar to that of factor VIII, except that since factor IX is distributed predominantly in the extravascular space, most of the injected dose will not be assayable in the blood. Recommended doses range from 15 to 40 units/kg depending on the clinical situation being managed. The dose of factor IX must be repeated about every 12 hours as for factor VIII. Hepatitis is no longer a major risk of transfusion of some factor IX concentrates [109].

Hypofibrinogenemia

Hypofibrinogenemia may occur as an isolated inherited deficiency or may be associated with obstetric complications, DIC, and some forms of cancer. In acquired hypofibrinogenemia, treatment should be directed toward the underlying cause of the disease rather than toward replacement of fibrinogen unless serious bleeding occurs. Many physicians provide fibrinogen replacement during correction of the underlying disorder. Usually a fibrinogen level of 50 mg/dL will prevent spontaneous hemorrhage, and 100 mg/dL allows adequate hemostasis following trauma or surgery [110]. Fibrinogen is available in cryopreceiptate or fibrinogen concentrate. Cryoprecipitate has been used as the source of fibrinogen for replacement therapy [93]. The dose of fibrinogen can be determined by either of the following methods: 100 mg fibrinogen per kilogram of body weight will raise the fibrinogen above 100 mg/dL [110]; or one bag of cryoprecipitate per 200 mL plasma volume will increase the fibrinogen to 100 mg/dL. Each bag of cryoprecipitate from a single donor contains approximately 200 mg of fibrinogen. The dose of fibrinogen for an adult is 6000–8000 mg, although this varies depending on the patient's fibrinogen level. Thus, usually about 30 bags of cryoprecipitate would be used. If cryoprecipitate is used as a source of fibrinogen, a quality-control program should be established by the blood bank so that its fibrinogen content will be known.

Fibrinogen

A virally inactivated fibrinogen concentrate prepared from human plasma is now available commercially and is approved by the FDA for the treatment of acute bleeding episodes in patients with congenital fibrinogen deficiency. The concentrate is recommended for use in these situations when the fibrinogen level is less than 100 mg/dL. The appropriate dose can be determined from the following formula: Target level (mg/dL) minus measured level (mg/dL) divided by 1.7. A single IV dose of 70 mg/kg body

weight increases the plasma fibrinogen level to approximately 130 mg/dL by 1 hour with a half life of approximately 96 hours [111]. While fibrinogen concentrate is approved only for use in congenital fibrin deficiency, small studies of its use in patients with low fibrinogen levels and massive bleeding from obstetric complications, cardiovascular surgery, intra abdominal surgery, trauma [112], and an aortic ascending aorta replacement [113] have suggested benefit.

Von Willbrand's Disease

Cryoprecipitate contains a substantial amount of the von Willebrand's factor from the original unit of blood [94, 114]. When replacement therapy is needed for these patients, multiple units of cryoprecipitate can be pooled. The usual dose of cryoprecipitate is one bag of cryoprecipitate per 10 kg body weight repeated every 8 hours. However, vWF concentrate is now available and is recommended instead of cryoprecipitate.

Deficiency of factor VII

This is very rare but now can be treated with activated factor VII.

Blood group compatibility of components used to replace coagulation factors

FFP need not be ABO identical but should be compatible with the recipient's red cells and can be given regardless of Rh type. Red cell compatibility testing is not necessary. Cryoprecipitate should also be administered as ABO compatible. Although the volume of each unit of cryoprecipitate is small, most therapy involves many units, and thus the total volume of plasma may be large. Cryoprecipitate can be administered regardless of Rh type. Cryoprecipitate and commercial concentrated preparations of factor VIII contain anti-A and anti-B, which may cause a positive direct antiglobulin test [115] and/or a hemolytic anemia if massive doses are administered. In addition, the recipient's fibrinogen may become elevated by the fibrinogen contained in cryoprecipitate if many units are given to patients who are not hypofibrinogenemic.

11.4 Transfusion of platelets

Platelet transfusion therapy has made major contributions to the care of a variety of patients. Platelets may be transfused either to prevent bleeding (prophylactic) or to treat active bleeding. The decision whether to transfuse platelets depends on the clinical condition of the patient, the cause of the thrombocytopenia, the platelet count, and the functional ability of the patient's own platelets. The responses to platelet transfusion vary, and the strategies to deal with patients who fail to respond are complex. Most platelets are transfused to patients with transient thrombocytopenia due to chemotherapy for malignancy, including hematopoietic stem cell transplantation (Table 11.11) [116–118]. Most

Table 11.11 Situations in which platelet transfusion may be necessary.

Decreased production
   Chemotherapy
   Aplastic anemia
   Irradiation
Increased destruction
   Disseminated intravascular coagulation
   Thrombotic thrombocytopenia purpura
   Cavernous hemangioma
   Autoimmune thrombocytopenia
   Drug-induced immune thrombocytopenia
   Neonatal alloimmune thrombocytopenia
Dilution
Massive transfusion
Platelet dysfunction
   Exposure to certain drugs
   Congenital platelet defects (name)
   Damage due to extracorporeal devices
     Extracorporeal membrane oxygenator
     Cardiopulmonary bypass instruments
   Metabolic effects
   Uremia

Source: Reproduced with permission from McCullough J. Transfusion medicine. In: Handin RI, Lux SE, Stossel TP, eds. Blood: Principles and Practice of Hematology. Philadelphia: J.B. Lippincott Company, 2003.

platelet transfusions are used for the prevention of bleeding rather than the treatment of active bleeding [116, 118].

The two kinds of platelet products are whole blood derived (Chapter 5) and apheresis platelets (Chapter 7). During the past decade, the use of apheresis has increased and in 2006 accounted for about 80% of the platelets transfused in the United States [119]. The reasons for this growth were thought to be to minimize the chance of (a) human leukocyte antigen (HLA) alloimmunization leading to platelet refractoriness, and (b) disease transmission. The TRAP study established that leukodepletion reduces alloimmunization and platelet refractoriness regardless of the platelet product used [39]. Apheresis platelets are necessary if the platelets are to be HLA matched or crossmatched with the recipient (see Management of Platelet Refractoriness), but this accounts for only 10–20% of transfusions. In most centers, apheresis platelets cost more than whole blood-derived platelets, and many centers do not believe this is cost effective. Thus, some/many centers use a pool of whole blood-derived platelets as the standard product and reserve apheresis platelets for those transfusions where all the platelets must come from the same donor (HLA and crossmatch).

Prevention of bleeding (prophylaxis)

Hemorrhage is the major cause of death in patients with bone marrow failure [120]. There is little risk of serious spontaneous hemorrhage when the platelet count is more than 20,000/mL, but the risk increases with lower platelet counts [116, 121]. Because of ethical concerns, very few controlled studies of prophylactic platelet transfusion were carried out when platelet therapy first became available. Several supported the use of prophylactic transfusions [122–124], but other studies were not able to show a benefit of prophylactic platelet transfusion [125, 126]. Despite the lack of substantial convincing clinical trial data, it became common practice for physicians to use platelet transfusions to prevent serious bleeding when the platelet count was less than 20,000/mL.

In an effort to better define the risks of thrombocytopenia, occult blood loss in the stools was quantitated using chromium-51-labeled red cells in patients with different degrees of thrombocytopenia (Figure 11.3) [127]. In stable thrombocytopenic patients, there was no increase in stool blood loss as long as the platelet count was above 5000/mL [127]. During the late 1970s and 1980s, considerable experience was gained in the management of thrombocytopenic patients, and this led to improvements in their outcome. Experience began to accumulate showing that patients could be maintained at platelet counts much lower than was previously believed to be possible. Serious bleeding usually occurred only when the platelet count was below 10,000/mL, and fatal bleeding is unlikely to occur at the platelet count above 5000/mL [121, 128–131]. A large number of studies have now
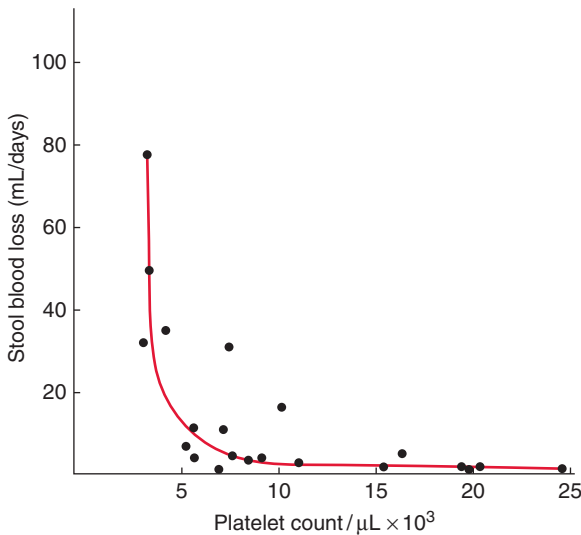
Figure 11.3 The relationship between stool blood loss and the platelet count as determined in 28 aplastic thrombocytopenic patients, according to Slichter and Harker. (Reproduced from Beutler E. Platelet transfusions: the 20,000/mL trigger. Blood 1993; 81:1411–1413.)

confirmed that a platelet count of 10,000/mL can be used safely in uncomplicated patients [129, 132–140]. An NIH consensus conference recommended that the 20,000/mL value traditionally used for prophylactic platelet transfusion could be safely lowered for many patients [141], and a recommendation by a prominent hematologist [142] began a major shift downward in the indications for prophylactic platelet transfusion. Now most physicians and hospital guidelines use platelet counts of 10,000/mL or 5000/mL as the indication for transfusion to uncomplicated patients.

The usual dose of platelets in a prophylactic platelet transfusion is 3–4 $\times$ 10^{11} platelets for an average-size adult. This usually involves a pool of four to six whole blood-derived platelets or one apheresis concentrate. There is no evidence that use of large doses of platelets prophylactically to maintain the platelet count at higher levels is necessary. Some patients with stable but low platelet counts, such as those with aplastic anemia or autoimmune thrombocytopenia, have little evidence of bleeding and do not require prophylactic platelet transfusions at all [127]. There seems to be an obligatory need for platelets to maintain endothelial integrity and, thus, hemostasis of about 7000 mL/day [143]. This, combined with the observation that spontaneous stool blood loss does not increase until the platelet count is less than 5000/mL, has led to proposals to decrease the usual platelet dose [144] in hopes of decreasing the need for platelets, although an economic model suggests that lower doses may increase the number of transfusions and the cost [145]. Others have shown that large numbers of platelets such as 8–16 $\times$ 10^{11} extend the time between transfusions (Chapter 7) [116, 146–148].

Two studies of platelet dose have been completed. The larger of these enrolled 1272 patients into three groups: the current standard dose, half, and double the current standard [116]. There was no difference in bleeding among the three groups demonstrating that smaller doses are as effective as the current standard dose for the prevention of bleeding. As expected, the post transfusion increments were lower in the low dose group and higher in the high dose group compared to the current standard. This in turn led to more frequent transfusions in the low dose group and fewer in the high dose group compared to the current standards. Another important result in this study is the observation that the likelihood of bleeding was no different for platelet counts ranging from 5000/μL to about 80,000/μL (Figure 11.4).

The other platelet dose study [148] was much smaller (119 patients) and compared the current standard dose of platelets with a dose of about one-half the current standard. Unfortunately, the study was stopped before completion and before reaching any statistical endpoint. This is because an arbitrary rule had been established that the study would be stopped if 50% of patients in either arm of the study experienced WHO grade 2 or greater bleeding which did occur in the low dose group. Thus, while this is concerning, the study size is too small and not statistically valid to provide definitive information. The previously mentioned larger study [116] probably has more clinical and certainly more statistical validity.
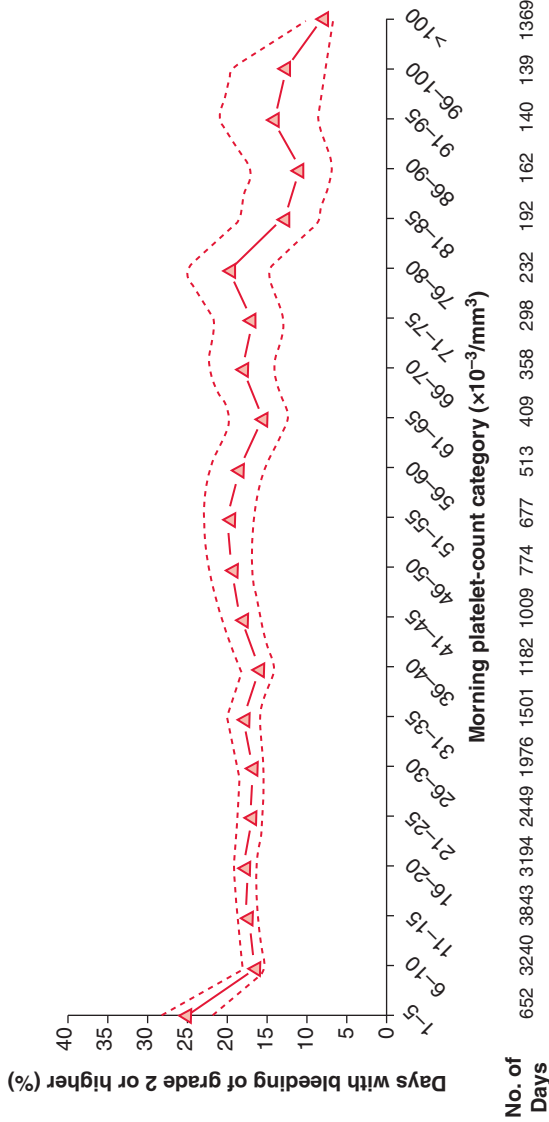
Figure 11.4 Days with bleeding of Grade 2 or higher in all three treatment groups, according to morning platelet count categories. (Reproduced with permission from Slichter SJ, Kaufman RM, Assmann SF, McCullough J, Triulzi DJ, Strauss RG, et al. Dose of prophylactic platelet transfusions and prevention of hemorrhage. N Engl J Med. 2010 Feb 18;362(7): 600–13 Copyright © 2010 Massachusetts Medical Society. All rights reserved.)
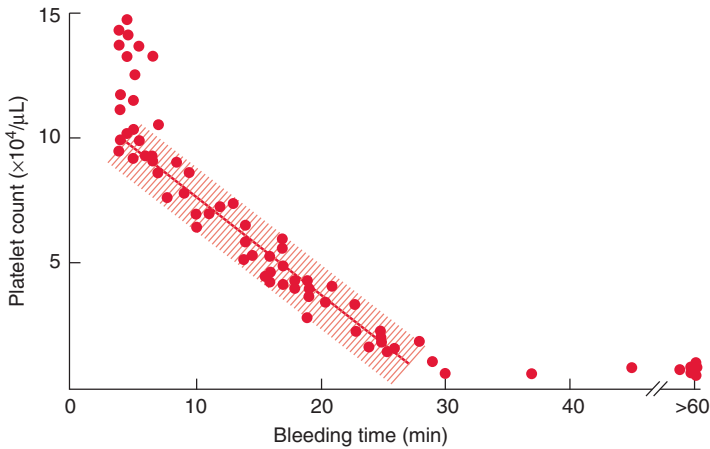
Figure 11.5 Inverse relation of bleeding time to circulating platelet count in patients with thrombocytopenia on the basis of impaired production when the concentration of platelets is between 10,000 and 100,000 per microliter. (Reproduced with permission from Harker LA, Slichter SJ. The bleeding time as a screening test for evaluation of platelet function. N Engl J Med 1972; 287:155. Copyright © 1972 Massachusetts Medical Society. All rights reserved.)

One other approach to prophylacticly reducing bleeding is the prevention of fibrinolysis and improving clot stability by administration of episilon aminocaproic acid [149, 150]. This appears to be useful and safe and is beginning to be used in practice.

Treatment of active bleeding

When considering platelet transfusion in a bleeding patient, data relating the bleeding time to the platelet count is helpful (Figure 11.5). With platelet counts below 100,000/mL, the bleeding time is increasingly prolonged, although it is only slightly prolonged when the platelet count is above 75,000/mL. Thus, platelet transfusions are not necessary for bleeding patients with a platelet count greater than 100,000/mL because they have a normal bleeding time [151]. The optimum platelet count to achieve in a bleeding patient is not known. In one study [152] of patients undergoing surgery, prophylactic platelet transfusions were given to those whose platelet count was less than 50,000/mL. Bleeding was similar in patients with counts less than 50,000/mL who received platelet transfusion and in those whose platelet count was greater than 50,000/mL but did not receive platelet transfusion. The bleeding did not relate to the platelet count, but instead to the severity of the surgical procedure. Thus, platelet transfusion should be considered in actively bleeding patients with a platelet count less than approximately 50,000/mL. An attempt to achieve a level greater than 50,000/mL is recommended. If the patient's platelets are dysfunctional, such as due to drugs or uremia, the bleeding time may be much longer than would be expected based on the degree of thrombocytopenia. In such situations, the decision to give a platelet transfusion is made on clinical

grounds alone. The bleeding time is also related to the hematocrit. Anemia is associated with a prolonged bleeding time [153]. For instance, in normal subjects a two-unit red cell donation that causes a 15% reduction in hematocrit and a 9% reduction in platelet count results in a 60% increase in the bleeding time [153]. Thus, giving a red cell transfusion to a severely anemic patient may also be beneficial in preventing or stopping bleeding.

In patients with autoimmune thrombocytopenic purpura, the bleeding time is also prolonged when the platelet count is less than 100,000/mL [151]. Spontaneous bleeding is not common in spite of the level of thrombocytopenia, possibly because the few platelets circulating are young and provide a shorter bleeding time than would be expected for that degree of thrombocytopenia [151]. These patients have platelet autoantibodies, and thus transfused platelets usually have a very short survival and limited effectiveness. Platelet transfusion may be effective in controlling serious active bleeding (e.g., during surgery), but is not recommended for prevention of bleeding. In patients with drug-induced immune thrombocytopenia, the offending drug should be discontinued and the patient closely observed. Because transfused platelets will have shortened survival, they are recommended in these cases only for treatment of severe thrombocytopenia with active hemorrhage.

Prophylaxis for invasive procedures

There are very few structured studies to provide data on the need for platelet transfusion in thrombocytopenic patients undergoing invasive procedures. Generally, a platelet count >40,000/μL seems to be adequate [128, 130, 131]. Bone marrow biopsy and lumbar puncture may be done safely with a platelet count of >20,000/μL [128]. Other recommendations are liver biopsy >50,000/μL, GI endoscopy >20,000/μL, bronchoscopy >20,000/μL, transbronchial biopsy >40,000/μL, and dental extractions and catheter insertions >50,000/μL [154].

Outcome of platelet transfusion

There is a dose–response effect from platelet transfusion (Figure 11.6) [121, 155]. Within 1 hour after transfusion, the platelet count increases by approximately 10,000/mL when 1×10^{11} platelets are transfused into a 70-kg patient [155, 156]. Since one unit of whole-blood-derived platelets usually contains approximately 0.7×10^{11} platelets, this should cause a platelet count increase of 5000–10,000/mL in an average-sized adult. Thus, in an adult with 1.8 m^2 body surface area, if it is desired to elevate the platelet count from 5000 to 40,000/mL, five units of whole-blood-derived platelet concentrate would be required: $(40,000 - 5000)/7500 = 5$. One plateletpheresis unit usually contains about 4×10^{11} platelets and thus could be expected to increase the platelet count about 40,000/μL. The 1-hour posttransfusion platelet count is an excellent predictor of an effective platelet transfusion [156]. If a very accurate determination of the response to platelet transfusion is needed, the 1-hour corrected count increment (CCI) or the percent recovery can be determined. The CCI is
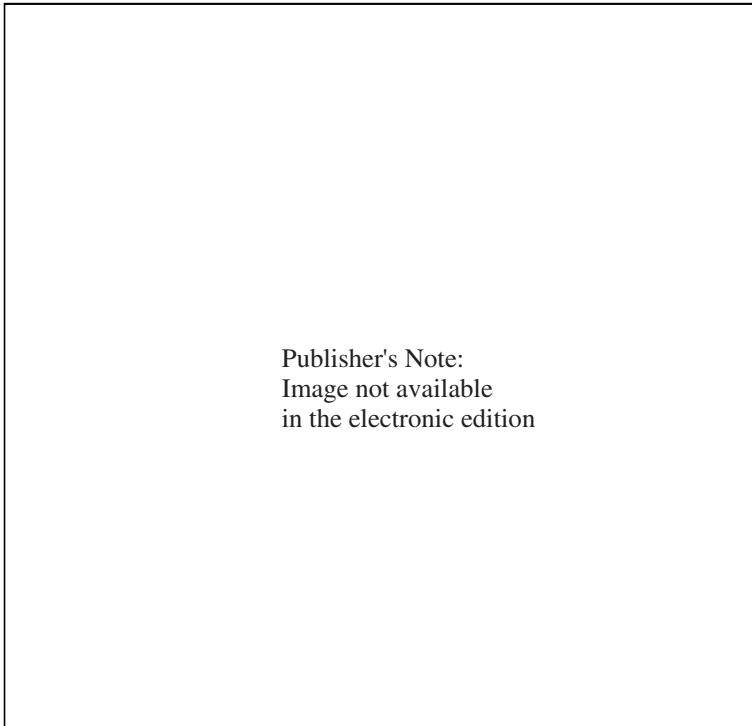
Figure 11.6 Effect of dose of platelets transfused on increment of platelet count. (Reproduced with permission from Freireich EJ, Kliman A, Gaydos LA, et al. Response to repeated platelet transfusion from the same donor. Ann Intern Med 1963; 59:277.)

most commonly used because it takes into account the size of the recipient and number of platelets transfused. The CCI is calculated as follows:

$$CCI = (Posttransfusion - Pretransfusion\ Platelet\ Count)$$
$$\times (Body\ Surface\ Area)/(No.\ of\ Platelets\ Transfused)$$

The expected CCI is about $15,000/mL/10^{11}$ platelets transfused per square meter of body surface area. If the CCI is less than 5000, the patient is considered to be refractory. If platelet recovery is used as an indicator, the expected result is about 65% because some of the platelets normally are sequestered in the spleen [157].

ABO and Rh in platelet transfusion

ABO antigens are on platelets. These antigens consist of type II chains intrinsic to the platelet and type I chains representing the soluble ABH antigens normally found in plasma and adsorbed onto the surface of the platelet [158]. When ^{51}Cr-labeled group A platelets are transfused to

normal group O volunteers, the recovery is reduced to one-third of that occurring following ABO-compatible transfusions [159]. The higher the ABO isoagglutinin titers, the greater the reduction in recovery of transfused platelets. This ABO effect was later substantiated clinically in several studies. Duquesnoy [160] reported a 23% reduction in recovery when platelets from HLA-matched donors were transfused to alloimmunized patients who were ABO incompatible with the donor platelets. Skogen et al. [161] reported two group O patients who were refractory to platelets transfused from group A donors but not from platelets transfused from group O donors. Heal et al. [162] reported a 41% decrease in the recovery of A- or B-incompatible platelets compared with ABO-compatible platelets transfused to HLA-matched refractory patients. Similar observations were also reported by Brand et al. [163] and in children by Julmy et al. [164]. Ogasawara et al. [165] also reported a patient in whom there was a poor response to some, but not all, ABO-incompatible platelets. The transfusions providing a poor response were from donors with high expression of A or B antigens. In a systematic review of 19 studies, the platelet count increment was consistently higher with ABO identical versus nonidentical platelet transfusions, although the largest difference in increment was only 4000 and so the clinical implication of these differences is open to question. No consistent effect on clinical outcome was apparent in these 19 studies [166]. All of these studies substantiate the concept that if platelets containing ABH antigens are transfused into patients with circulating antibody directed against those antigens, the intravascular recovery of the transfused platelets is substantially decreased and Kaufman. In platelet transfusion, the ABO blood group system does matter [167–170].

Another aspect of platelet ABO incompatibility makes this situation even more interesting. Heal et al. [162] also observed an 18% decreased recovery of transfused platelets when the incompatibility involved transfusion of ABO antibody directed against the recipient's ABO antigens (e.g., group O platelets transfused to a group A patient). It is postulated that the ABO effect is due to the formation of circulating immune complexes by soluble ABO substance and ABO antibodies [171]. This can occur with the antigen–antibody complexes from either donor or recipient antibody and donor or recipient ABO substance. Thus, the reduced platelet recovery due to circulating immune complexes can occur when the ABO incompatibility is either the "major" or "minor" type [168].

The results of the Duquesnoy study [160] have been interpreted by some to mean that ABO incompatibility need not be considered in platelet transfusion. However, the specific conclusions of those authors dealt with whether ABO incompatibility should preclude the use of ABO-incompatible HLA-matched platelets for refractory patients. They concluded that, although there was a definite effect of ABO incompatibility in reducing posttransfusion survival, this was not of a magnitude that would contraindicate transfusion of ABO-mismatched but HLA-matched platelets. The emphasis, however, was on the overriding value of HLA matching but that ABO incompatibility definitely reduced the effect of the

transfusion. Thus, it is now clear that ABO incompatibility is associated with reduced posttransfusion platelet recovery.

A separate consideration involving the ABO system and platelet transfusion is the potential administration of large volumes of ABO-incompatible plasma if transfusions involve "minor" incompatibility (e.g., group O platelets transfused to a group A patient). As described above, it appears that this may reduce the survival of the transfused platelets, but another concern is the potential for hemolysis when large amounts of ABO-incompatible antibody are transfused. This has been reported [172–174], and it is now common to limit the volume of ABO-incompatible plasma a patient can receive. This can be in terms of a percentage of the patient's estimated blood volume or an absolute volume limit. For instance, an adult patient could be limited to no more than 1 liter of ABO-incompatible plasma per week. Alternatively, an ABO titer screening can be done on apheresis units—usually using a cutoff of 1:50, although there is no data supporting this value.

Rh antigens are not present on the platelet surface. However, the few red cells contained in the platelet concentrate can lead to immunization, and thus Rh must be considered in platelet transfusion. In Rh-negative oncology patients, reported rates of immunization to the D antigen range from 0% to 18% [175–179]. Because oncology and bone marrow transplant patients receive a large number of platelet transfusions, it is usually not possible to provide all Rh-negative platelets for Rh-negative patients. Thus, it is common to provide platelets to these patients regardless of Rh type. $Rh_o(D)$ immune globulin (RhIG) is not administered to prevent alloimmunization in these patients. Even when patients develop circulating anti-D, this antibody does not interfere with the circulation of Rh(D)-positive platelets [180]. However, Rh-negative platelets are recommended for patients who can be expected to receive only a few platelet transfusions and who may have long-term survival with the potential to become pregnant in the future. Examples of such patients are women of childbearing age undergoing cardiovascular or orthopedic surgery or experiencing acute trauma or complications of pregnancy. If Rh-negative platelets are not available, transfusion should not be delayed. Instead, RhIG can be administered to prevent alloimmunization. The dose of RhIG can be determined from the number of units of platelets that patient receives. For instance, since most therapeutic doses of platelets (e.g., one single donor unit, or six pooled whole blood-derived donor units) contain less than 1 mL of red cells (see Chapter 5), one standard dose of 300 mg of RhIG is sufficient.

Lack of response to platelet transfusion (refractoriness)

Many patients do not attain the expected posttransfusion increment in platelet count; these patients are said to be "refractory" to platelet transfusion. This is one of the major problems in platelet transfusion. Refractoriness to platelet transfusion can be caused by many factors, some related to the patient and some related to the platelet concentrate. Another way of categorizing the reasons for refractoriness is immune versus

nonimmune factors. It is often difficult to separate these, but in one study nonimmune clinical factors were present alone in 67% of patients and coexisted with immune factors in an additional 21% of patients [181].

In the past the incidence of refractoriness was rather high; in one study, about 38% of patients [182]. It appears that patients who become refractory do so after relatively few transfusions, and some patients do not become refractory regardless of the number of transfusions [182]. A later study found only about 15% of patients became refractory after the first 8 weeks of therapy [39]. This may be a result of changed transfusion practices (described in Section "Prevention of alloimmunization and platelet refractoriness").

Factors related to the patient

In patients who have platelet antibodies, such as those with autoimmune thrombocytopenic purpura or patients who are immunized to antigens of the HLA system, survival of circulating platelets is extremely brief, sometimes only a matter of minutes [126]. Splenomegaly also causes sequestration of platelets and a reduced posttransfusion increment. Other patient-related factors associated with a reduced platelet transfusion response have been reported in three studies (Table 11.12). In the largest study [39] of 6379 transfusions to 533 patients, 12 different factors were identified and similar but fewer factors were seen in the two smaller studies [181, 183]. Thus, patients can be refractory because of alloimmunization

Table 11.12 Patient-related factors associated with reduced response to platelet transfusion.

	Authors		
Condition	Slichter (185) 533[a]–6379[b]	Bishop (183) 133[a]–941[b]	Doughty (181) 26[a]–266[b]
Less than two pregnancies	X		
Male gender	X		
Splenomegaly	X	X	
Bleeding	X		X
Fever	X	X	X
Infection	X		X
DIC	X	X	X
? Height and weight	X		
HLA antibodies	X	X	
Platelet antibodies		X	
? Number of platelet transfusions	X		
Heparin receipt	X		
Amphotericin receipt	X	X	
Marrow transplant		X	
Antibiotics receipt			X

[a]Patients.
[b]Transfusions.

(HLA and/or platelet antibodies) or nonimmune factors. The exact proportions of refractory patients resulting from alloimmunization and nonimmune clinical factors is not known. Many patients who have HLA antibodies are not refractory and in, most studies, refractoriness is clearly associated with the presence of HLA antibodies [156, 184], although there is not a generally accepted laboratory test that defines with high precision refractoriness due to alloimmunization. Also, many of these patients have one or more clinical factors present that could account at least partly for the refractoriness. Thus, in practice it is very difficult to determine whether a patient is refractory due to immune or nonimmune factors, and this affects the strategy used to manage these patients.

Factors related to the platelet concentrate

ABO incompatibility reduces the intravascular recovery and survival of transfused platelets. Because ABO compatibility improves platelet response [159–170, 185], patients who are refractory to platelet transfusion should receive a trial of at least two ABO-identical platelet transfusions. Another cause of reduced recovery of transfused platelets is the transfusion of platelets near the end of their storage period. Lazarus et al. [186] demonstrated that platelets stored for longer than 24 hours provided a substantially reduced posttransfusion increment, and Slichter et al. [185] found that platelets stored for less than 48 hours gave better responses than older platelets. Although there are no extensive data on this topic, it is generally accepted that platelets near the end of their 5-day storage period may provide less of an increment than fresh platelets; therefore, in determining whether a patient is refractory, at least one transfusion of platelets 24–48 hours old should be provided.

Strategies for managing patients refractory to platelet transfusion [187]

HLA matching for platelet transfusion

If the use of ABO-matched platelets of less than 48 hours' storage fails to produce a satisfactory increment, efforts should be made to use platelets that are HLA-matched to the recipient. During the 1960s it was observed that in refractory patients, platelets from HLA-identical siblings provided a good response [188]. Later it was shown that HLA-identical platelets from unrelated donors were also beneficial [189]. This still provided matched platelets for only a limited number of patients because of the small chance of finding an HLA-identical donor in the random population. The use of HLA-matched platelets became practical when it was shown that good responses could be obtained when the donor platelets were only partially matched (Figure 11.7) [190–194]. Many blood banks have large files of HLA-typed volunteer donors so that HLA-matched platelets can be located for most patients. However, most HLA-matched platelets obtained from unrelated donors have some antigens mismatched with the patient. Although the average response to these partially matched transfusions is similar to that from fully matched, HLA-identical transfusions, 20–50% of HLA-matched transfusions do not provide a satisfactory response [193].
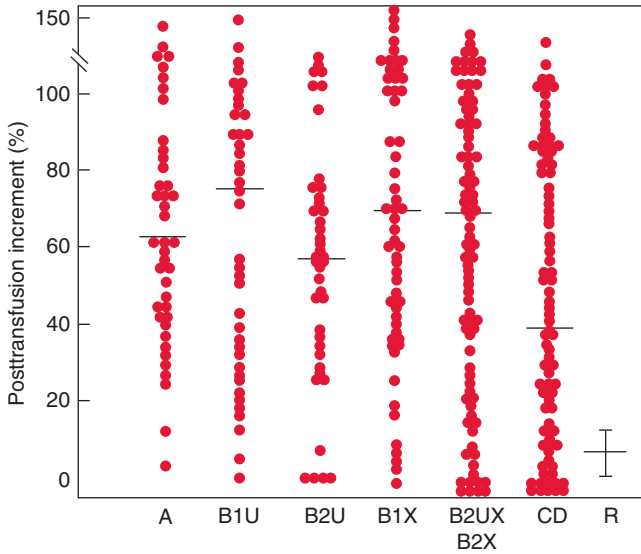
Figure 11.7 Platelet increments in alloimmunized thrombocytopenic patients 1 hour after transfusion of platelets from donors of varying degrees of HLA compatibility. "R" indicates response to pooled, random platelets (ave. ± 1SD). Horizontal bars indicate median response. (Reproduced from Duquesnoy RJ, Filip DJ, Rodey GE, et al. Successful transfusion of platelets "mismatched" for HLA antigens to alloimmunized thrombocytopenic patients. Am J Hematol 1977; 2:219.)

Also, since these patients require frequent platelet transfusions, many donors who are matched to the patient must be available to sustain the patient for days or a few weeks [195].

Crossmatching for platelet transfusion

Because HLA-matched transfusions have a 20–50% failure rate, crossmatching the patient's serum and the potential donor's cells has been used. The development of this approach was slow for many years because the platelet antibody detection techniques were complex and did not lend themselves to rapid response on a large scale with relatively low cost. While it appears that in most patients HLA antibodies are responsible for refractoriness, platelet-specific antibodies may sometimes be involved. Because the relative roles of platelet-specific and HLA antibodies were not clear, this further complicated the choice of the optimum method for crossmatching [196].

There has been great variability in the experience with platelet crossmatching [193]. The positive predictive value of platelet crossmatching ranged from 73% to 100% and the negative predictive value from 52% to 92% in 10 studies summarized by Heal et al [162]. Many of these studies were retrospective, and some of the prospective studies did not use fresh platelets. Good results have been obtained with a monoclonal anti-IgG assay [196], a platelet migration inhibition assay [197], platelet

immunofluorescence [198], and microtiter platelet crossmatch [162, 199]. Unsatisfactory methods include granulocytotoxicity, microleukoagglutination, capillary leukoagglutination were not helpful [197], serotonin release, platelet factor 3 release, and platelet aggregometry [200]. Moroff et al. [193] compared HLA matching and platelet crossmatching in 73 patients. Although platelet refractoriness is often associated with the presence of lymphocytotoxicity antibodies in the patient's serum, Moroff et al. [193] were unable to demonstrate lymphocytotoxic antibodies in 55% of the patients in their study who were refractory to platelet transfusion and had no obvious clinical causes for the refractoriness. Both HLA matching and platelet crossmatching provided similar degrees of successful 1-hour CCIs (40–60%). If transfusions only with the HLA matching of grade A or Bu were considered, HLA matching was superior to platelet crossmatching. However, Moroff et al. [193] concluded that a similar number of successful transfusions could be obtained by either HLA matching or platelet crossmatching.

A solid-phase red cell adherence assay for platelet crossmatching [201], now in clinical use, predicted a successful transfusion outcome in 97% of patients with no clinical factors to cause nonimmune platelet destruction [202]. Successful transfusion response in a separate study [203, 204] of 962 single-donor platelet transfusions to 71 refractory patients showed that the solid-phase red cell adherence assay was superior to HLA matching. This method is now used routinely because of its effectiveness and practicality.

Other approaches to the refractory patient

Other strategies that have been attempted include plasma exchange [205], treatment of the patient's plasma with a staphylococcal protein A column [206], and administration of cyclosporine. These methods are not usually successful and are not recommended. Case reports have shown a beneficial effect of intravenous immunoglobulin in improving the response to platelet transfusion in alloimmunized refractory patients [207, 208], but another larger, more definitive study found no benefit [209] and recommended against its use.

A practical approach to the patient refractory to platelet transfusion

The lack of a response to platelet transfusion is often associated with bleeding. These patients are usually quite ill with problems such as fever, sepsis, disseminated intravascular coagulopathy, or viral infections. Whether patients with similar clinical problems but who are responsive to platelets have fewer bleeding problems has not been established. However, because refractoriness is often associated with bleeding and a poor outcome, it is important to define the steps that can be taken to improve the response to transfusion in these patients.

Clinical factors, such as infection that might cause refractoriness, should be sought and, if present, appropriate treatment should be initiated to

Table 11.13 Practical strategies for dealing with platelet refractory patient.

1. Treat any correctable clinical factors present that may cause platelet refractoriness. Until these factors are eliminated, recognize that any of the steps listed below may not be effective.
2. Ensure that the patient is receiving the correct dose of platelets.
3. Give at least one test transfusion of platelets that are not more than 48 hours old.
4. Ensure that platelets being transfused are ABO identical.
5. If these steps have failed and transfusion is urgently needed, give transfusions of either crossmatch-compatible or HLA-matched platelets, whichever is available soonest. Give at least one and preferably two or three such transfusions.
6. Continue to use either crossmatched or HLA-matched platelets until the desired increment is obtained.
7. Determine whether the patient has HLA or platelet-specific antibodies or both. This may be of value in making future decisions about platelet transfusion if the patient does not respond to HLA-matched or crossmatched platelets.

correct them [209a]. Additional simple steps that should be attempted (Table 11.13) include the transfusion of ABO-identical platelets and platelets less than 48 hours old. The techniques for platelet transfusion should be reviewed to be sure the platelets are not being damaged or lost because of improper handling after leaving the blood bank, use of incorrect filters, or improper storage conditions after leaving the blood bank. An additional step is to be certain that the platelet concentrates contain an adequate number of platelets. This can be done by checking the routine quality control testing that the blood bank carries out. If questions continue, the platelet count and content of the specific concentrates being used for the patient can be determined. If these measures fail, platelets that are matched to the recipient should be used. This can be accomplished by HLA matching donor and recipient or by using platelets that are compatible in a crossmatch. As described above, the success rate is similar for HLA-matched or crossmatch transfusions. One practical difference is that usually crossmatched platelets can be obtained more rapidly than HLA-matched platelets because crossmatching is done on platelets already collected and available in inventory, whereas HLA-matched donors must be located and scheduled for donation.

Some patients do not respond to either HLA-matched or crossmatched platelets. Often these are patients in whom marrow grafting has failed or who have GVHD, are septic, or are experiencing other severe complications of cytopenia (Table 11.13). The transfusion strategies for these patients should be based on a discussion between the patient's attending physician and the transfusion medicine physician. One approach is increasing the dose of platelets to two or even three single-donor or 20–30 random-donor units per day. While this may enable the physicians to feel that they are doing something helpful, usually the patients fail to achieve a substantial increase in platelet count. It is not known whether these additional transfusions are helpful despite the lack of increase in circulating platelets.

Reducing the volume of the platelet concentrate

The dose of platelets necessary in some situations may result in a volume of plasma in the platelet concentrate that is too large for the patient to safely tolerate. Examples of these situations are transfusion to small children or the need for large doses of platelets to average-sized adults. In such situations, the platelet concentrate can be centrifuged and excess plasma removed safely anytime during the 5-day storage period [210]. It is important that the centrifugation technique be gentle and that the laboratory gain some experience with the technique to ensure that there is not excessive damage to platelets before attempting this for patient therapy.

Prevention of alloimmunization and platelet refractoriness

Because of the difficulty in managing patients who are refractory to platelet transfusion and the role of alloimmunization in refractoriness, there is considerable interest in preventing alloimmunization. Immunization may occur due to pregnancy but is also thought to be caused by the leukocytes contained in the platelet and red cell transfusions patients receive [211, 212]. The strategies that have been attempted include limiting the number of donor exposures, removing the leukocytes, or treating the components to render the leukocytes nonimmunogenic. Gmur et al. [213] demonstrated that use of non-HLA-matched single-donor platelet concentratescompared with pooled whole blood-derived platelets reduced the incidence of alloimmunization (HLA), prolonged the time to immunization, reduced the incidence of refractoriness, and prolonged the time to refractoriness. Clinical trials are not perfect and many of these studies have one or another shortcoming; however, there is little disagreement that leukodepletion reduces the incidence of allimmunization ad delays and/or reduces the onset of refractoriness [36–39, 213–218]. In the largest prospectively randomized trial reported [39], more than 500 newly diagnosed acute leukemia patients, the use of leukodepleted red cells and platelet concentrates reduced the incidence of alloimmunization. There was no difference between pooled random-donor and single-donor platelet concentrates, suggesting that the important factor is the reduction of the leukocyte exposure rather than the number of different donors. Although studies never clearly established whether bedside leukodepletion could prevent alloimmunization and refractoriness, the issue is moot because prestorage leukodepletion is used increasingly because it (a) prevents accumulation of cytokines that cause transfusion reactions [219–221], and (b) is more standardized and reproducible.

Another strategy proposed for the prevention of alloimmunization is the treatment of the platelet concentrates with ultraviolet light. Ultraviolet radiation inhibits the ability of lymphocytes to either proliferate or stimulate in a mixed lymphocyte culture (MLC) [222] but UV-B light does not interfere with platelet function in vitro [222, 223] or in vivo [224] at doses that will abrogate lymphocyte function. In mice, UV-B treatment of

platelets reduced the alloantigenicity of transfused leukodepleted platelets [225], and nonleukodepleted transfused platelets [226]. In the TRAP study, UV-irradiated platelets reduced the incidence of alloimmunization to the same extent filtered leukodepleted platelets [39]. Thus, although this approach appears to be effective in preventing alloimmunization, it was never developed commercially. Pathogen inactivation techniques (see Chapter 5) involve treatment of platelets with UV light and may reduce alloimmunization [227, 228].

11.5 Granulocyte transfusion

As the granulocyte count falls below 1000/mL, there is an increased risk of infection [229], which is further increased by the duration of granulocytopenia [230]. The increasingly common and aggressive treatment of patients with hematologic malignancies and aplastic anemia has made granulocytopenia common. During the early 1970s, almost 80% of deaths in patients with bone marrow failure were due to infection [229]. Because of the success of platelet transfusion in the management of hemorrhage due to thrombocytopenia, there was considerable interest in transfusion of granulocytes for the management of infection in granulocytopenic patients. Because of the small number of granulocytes in the peripheral blood of normal humans, collection of cells for transfusion was not practical. Initial studies of granulocyte transfusion involved cells obtained from patients with chronic myelogenous leukemia. During the late 1960s and early 1970s, several blood cell separators were devised to allow collection of approximately 4×10^{10} granulocytes from patients with chronic myelogenous leukemia and 1×10^{10} granulocytes from normal donors by leukapheresis (Chapter 7) [231]. These cells function normally in vitro and have normal in vivo survival and migration to sites of infection [231–233]. Granulocytes for transfusion have also been prepared by isolating the buffy coat from units of fresh whole blood. This has usually been done for transfusion of neonates (see Chapter 12) because they do not require the larger dose of cells produced by leukapheresis. However, there is little experience with this technique; no large clinical studies of its use have been reported and it is not recommended.

A series of studies in the 1970s established that granulocyte transfusion provided improved survival in patients with documented gram-negative sepsis who remained granulocytopenic for at least 10 days [234–237]. Some additional data indicated that granulocytes may be helpful in patients with other kinds of documented infections (either Gram positive or Gram negative) and granulocytopenia of more than 10 days' duration [237], but there is little information regarding other organisms. Patients with fever of unknown origin do not experience improvement as a result of granulocyte transfusion [235]. There is also little information that collates the site of infection other than bacteremia with response to granulocyte transfusion [234]. Currently most patients respond to antibiotics, and granulocyte transfusion is rarely used for bacterial infections.

There is no clear description of patients eligible for granulocyte transfusion. Ideally, these are expected to be neutropenic for several days and have proven infection unresponsive to appropriate antibiotics. However, in practice often patients have clinical signs and symptoms of serious infection but no positive cultures. Also, many of the patients who do not respond to appropriate antibiotics have severe (less than $100/\mu L$) and prolonged neutropenia. Fever may be due to documented or assumed fungal infection [238–241], which requires anti-fungal therapy. When signs and symptoms of infection persist in these patients, granulocyte transfusions are often used. No clinical trials have documented the effectiveness of granulocyte transfusion for fungal infections, although animal [240] and clinical [241] studies support their use, and granulocytes do kill fungi in vitro.

The minimum dose of granulocytes required for clinical efficacy has not been established. Early clinical trials involved about 1×10^{10} granulocytes per day. However, in the decades since, antibiotics and the management of neutropenic patients have improved and so this dose is no longer adequate. The minimum number of transfusions necessary for improvement differs depending on the patient's clinical status. Granulocyte transfusion should be used as a course of treatment similar to antibiotics. Monitoring clinical symptoms and signs, such as temperature elevation, as a means of deciding daily whether to use granulocyte transfusions can be misleading and will create logistical problems with procurement of granulocytes. Just as antibiotics would not be discontinued on the basis of brief clinical improvement, granulocyte transfusions once initiated should be continued daily until the patient's general condition has stabilized and improved. In general, it is advisable to consider a course of granulocyte transfusion therapy as lasting at least 7 days.

Most experience with granulocyte transfusion involves treatment of existing infection. At one time, there has been considerable interest in the use of granulocytes to prevent infection in granulocytopenia patients. However, prophylactic granulocyte transfusions are not helpful in this regard for newly diagnosed patients with acute myelogenous leukemia [242].

Granulocyte colony-stimulating factor (G-CSF) have been used to stimulate granulocyte production in neutropenic patients. Stimulation of normal blood donors with G-CSF increases the level of circulating granulocytes and increases the yield of granulocytes [243–246]. By combining the use of corticosteroids and G-CSF, the level of circulating granulocytes can be increased further to about 40,000/mL, compared with about 30,000/mL with G-CSF alone (see also Chapter 7) [245, 246]. This can provide a dose of up to 8×10^{10} granulocytes or eight times the doses used in the studies of the 1970s [245, 247, 248]. The availability of these strategies to obtain substantially greater granulocyte doses for transfusion is stimulating renewed interest in clinical trials of granulocyte transfusions and a large multi-center clinical trial is underway [249–253].

Granulocyte transfusion of septic neonatal patients is described in Chapter 12.

CMV can be transmitted by blood transfusion with a severe, even fatal result. Most individuals previously infected with CMV are no longer infectious but they have antibodies to CMV. Unfortunately, there is no simple, effective test that can be used to distinguish CMV antibody-positive blood donors who are infectious. Providing blood components that do not contain anti-CMV is a well-established method of preventing transfusion-transmitted CMV disease (see below). Because a substantial portion of blood donors are CMV antibody positive, providing CMV antibody-negative blood components is sometimes difficult, especially as the indications for CMV-free blood components have increased. Attempts to improve the testing methods to identify only the infectious units have not been successful. Alternatively, because it is presumed that leukocytes are the reservoir of CMV in asymptomatic blood donors, prevention of transfusion-transmitted CMV has been attempted using leukodepleted components (see section on leukodepleted red cells). Leukodepleted and antibody-negative components can be considered equivalent and used interchangeably depending on logistical considerations of each transfusion service (see Chapter 5). This section describes the indications for CMV-free blood components in different situations. The increasing use of leukodepleted red cells is making some of this discussion less important, since all patients may be receiving leukodepleted red cells regardless of their specific risk for CMV transmission.

Neonates

Landmark studies by Yaeger and colleagues [254] established that neonates weighing less than 1200 g born to CMV-negative mothers were at substantial risk of developing CMV disease if they received blood from CMV antibody-positive donors. Serious disease or death occurred in 50% of the infected neonates. These observations have been supported by some other studies but not by others. This is not surprising because of the large variation in the prevalence of CMV in blood donors in different parts of the United States. It has been suggested that at least some of the differences between the studies mentioned above could be a changing incidence of transfusion-transmitted CMV infection due to differences in techniques of CMV testing of donated blood, less use of fresh blood components, more use of plasma containing CMV antibodies, and a changing donor population because of HIV and non-A, non-B hepatitis screening practices. Despite these differences, CMV safe blood is usually used for neonates.

Pregnant women

CMV infection of the fetus is a severe clinical problem that can cause growth and mental retardation, hepatitis, deafness, intracranial calcifications, and bleeding diathesis. Congenital CMV infection due to primary infection in the mother is usually more serious than infections due to recurrent maternal CMV infection [255]. Thus, it is prudent to avoid

primary CMV infection in pregnant women. Although there have been no specific clinical studies of this situation, it is advisable to provide CMV-free blood to pregnant women or for intrauterine transfusion of the fetus [256].

Kidney transplantation

CMV disease is common in renal transplant recipients, occurring in 60–90% of patients. Because it can be very serious, causing graft rejection or even death, its prevention is important. The cause of CMV disease in these patients may be (a) reactivation of latent previous CMV infection; (b) acquisition of CMV from the donor organ; or (c) acquisition of CMV from blood transfusion. The prevalence of CMV ranges from 30% to 70% in the blood donor population [257], and most renal transplant patients will have received previous transfusions, further increasing the proportion of them who have previous CMV infection. Thus, reactivation of a previous infection is by far the most common cause of CMV disease in these patients [258, 259].

Approximately 77% of CMV antibody-negative patients who receive a kidney from a CMV antibody-positive donor develop CMV infection, compared with 8–20% in similar patients who receive a kidney from a CMV antibody-negative donor but receive CMV antibody-positive blood transfusions [258, 259]. If a CMV antibody-negative patient receives a kidney from a CMV antibody-negative donor and then receives CMV antibody-negative blood components, the likelihood of developing CMV infection is almost zero [259]. Thus, it is advisable to provide CMV-free blood components for these latter patients.

Renal transplant patients with anti-CMV may develop CMV disease due to acquisition of a second strain of the virus from the donor organ [260]. Acquisition of a second strain of CMV from blood transfusion has not been reported. Thus, providing CMV-free blood components for patients with anti-CMV to avoid transmitting a new CMV strain is not presently recommended.

Bone marrow transplantation

CMV disease is a major complication of marrow transplantation. CMV disease is a more frequent and severe problem in marrow transplantation patients because they are more severely immunosuppressed than solid organ recipients. CMV disease occurs in 40–50% of patients undergoing allogeneic marrow transplantation, and it is an important source of posttransplant mortality [261]. As with renal transplantation, the majority of CMV disease in marrow transplantation patients occurs in patients with CMV antibody and is probably due to reactivation of virus from a previous infection, not to acquisition of a new strain [262]. However, in CMV antibody-negative patients who receive marrow from a CMV antibody-negative donor, the risk of developing CMV infection is approximately 40% [262]. The likelihood of these patients acquiring CMV infection is almost eliminated by the use of CMV antibody-negative blood or leukocyte reduced components [42, 45, 46]. Thus, it is customary to provide CMV-free blood components to CMV antibody-negative marrow

transplantation patients whose marrow donor is CMV antibody negative. Providing CMV antibody-negative blood components for marrow transplantation patients may be difficult because of the large number of transfusions they receive, so leukocyte-depleted blood components are often used.

Since most CMV disease occurs in marrow transplant patients who are CMV antibody positive, extension of the use of CMV antibody-negative blood components to potential marrow transplant patients who are CMV antibody negative has been suggested. Thus, they would not become infected with CMV as a result of transfusions received earlier in their disease and before entering a marrow transplantation program. There are no clinical studies of this issue, but the increasing use of leukodepleted components is helpful as a way of providing the large number of CMV safe blood products for these patients.

Heart, heart–lung, liver, and pancreas transplantation

Very few data are available describing CMV infections in recipients of heart, heart–lung, liver, or pancreas transplantations. One small study of heart transplant patients demonstrated that CMV infection was transmitted by either the donor heart or blood components [263]. When both the donor organ and all blood components were CMV antibody negative, CMV infection was prevented in heart [263] as well as in liver [264] transplant recipients. Although the incidence of CMV infection in heart transplants may be very low (1.5%) [265] and data are not extensive and are somewhat conflicting for these other transplant situations, the patients are immunosuppressed, and CMV infection is a clinical problem that can be prevented by the use of CMV-safe blood components. This practice is advisable in CMV antibody-negative patients who receive an organ from a CMV antibody-negative donor. CMV infections in CMV antibody-positive heart transplant patients are the result of reactivation of latent virus [266], and therefore the use of CMV-free blood components is not indicated for these patients.

Acquired immune deficiency syndrome

Acquired immune deficiency syndrome (AIDS) patients have severely impaired immune function, and CMV infections are an important clinical problem for them. Thus, use of CMV-safe blood components is indicated for CMV antibody-negative AIDS patients, although no clinical trials have been done to form the basis for this practice. The same potential extension of the use of CMV antibody-negative blood components mentioned for potential marrow transplant patients also applies to individuals infected with HIV-1. That is, use of CMV-safe blood components is recommended for CMV antibody-negative patients infected with the HIV virus but who are currently asymptomatic. This would prevent the development of latent CMV infection in these patients, which could reactivate later when AIDS develops. The cost-effectiveness and practicality of this approach have not been established, but this is not a major issue because patients at this stage

of HIV infection usually do not need transfusions and, if they do, leukodepleted products are readily available.

Severe combined immune deficiency (congenital)

There are no specific studies or data regarding prevention of CMV infection in patients with congenital severe combined immune deficiency by the use of CMV antibody-negative components. However, because these patients are severely immunocompromised, provision of CMV-free blood components is the usual transfusion practice.

Patients receiving extensive chemotherapy

As more varied chemotherapy regimens are used, patients are experiencing greater drug-induced immunosuppression. However, in general, CMV infection has not been an important clinical problem in these patients. Since there is no good way of determining the level of immune function at which patients become increasingly susceptible to CMV infection, the use of CMV-free blood components is not recommended in these patients.

11.7 Irradiated blood components

Graft-versus-host disease

GVHD is a serious, often fatal disease that occurs when there are histocompatibility differences between the donor and recipient, the recipient receives immunocompetent donor cells, and the recipient is unable to reject the donor cells. GVHD was originally identified in immunodeficient children [267–270], but the more common situation in which GVHD occurs is in bone marrow transplantation [271]. It is now clear that GVHD can occur in a wide variety of immunocompromised patients and even in immunocompetent patients. In marrow transplantation, GVHD is caused by the immunocompetent marrow donor cells [271], whereas in blood transfusion, viable lymphocytes contained in blood components can cause fatal GVHD in susceptible patients. The hallmark features of GVHD are involvement of skin, liver, gastrointestinal tract, bone marrow, and lymphoid system [272]. There are some clinical differences between GVHD associated with marrow transplantation and transfusion-associated GVHD.

The majority of cases of GVHD occur in immunocompromised patients such as those with congenital immunodeficiency, those undergoing stem cell transplantation, or those who have received extensive myeloablative therapy [273–277]. However, cases of transfusion-associated GVHD have been reported in apparently immunocompetent patients [278–283]. It appears that this occurs when the donor is HLA homozygous and haploidentical with the recipient. In this situation, although the recipient may be immunocompetent, the donor cells would not be recognized as foreign and thus would not be rejected. If the donor cells are immunocompetent, they proliferate, causing transfusion-associated GVHD. There is a very high incidence (approximately 1 : 600) of

transfusion-associated GVHD in patients undergoing heart surgery in Japan [283, 284]. This has been attributed to the high incidence of HLA homozygosity in the Japanese population.

Transfusion-associated GVHD has also been reported when patients receive transfusions of fresh components such as platelets from first-degree relatives [278, 282]. Since there is a higher likelihood that these relatives will share an HLA haplotype with the patients, this same mechanism of an HLA-homozygous donor who is haploidentical with the patient has been proposed to account for these cases of transfusion-associated GVHD in immunocompetent patients.

Irradiation of blood components

To prevent transfusion-associated GVHD, blood components are subjected to radiation, which interferes with the ability of lymphocytes to proliferate. Blood components can be successfully irradiated using either gamma radiation or x-rays. Both of these methods damage the lymphocytes by forming electrically charged particles or ions that alter the DNA, making the lymphocyte unable to proliferate [285]. Hospitals that provide very few irradiated units may do this using x-ray instruments; 13.5% of hospitals in an AABB survey. Most [80%] blood banks that provide large numbers of irradiated products usually do this with a dedicated instrument. These instruments usually use ^{137}Cs, although a few use ^{60}Co as the source of the radioactivity. These blood irradiators may contain different amounts of isotope, and this affects the length of time required to irradiate the blood components. Also, different irradiation devices contain radiation chambers of different sizes, and thus there are differences in the number of units of blood components that can be irradiated.

Cesium is used as a water-soluble salt (cesium chloride) that is highly dispersible which has led to security concerns since most of these irradiators are in nonsecure areas of hospitals. The National Academy of Sciences has recommended decommissioning the devices, although no such regulatory action has been initiated by the Nuclear Regulatory Commission [286].

The optimum minimal dose of radiation necessary has evolved over the years. Following early experiences with transfusion-associated GVHD, a minimum dose of 3000 rads was recommended [268]. The occurrence of transfusion-associated GVHD in several individuals who received blood components supposedly irradiated with at least 1500 cGy [273, 287, 288] has focused attention on the configuration of the blood containers in the irradiated field, the distribution of radiation within the field, and quality control methods to ensure that the desired dose was actually being administered [285].

Most institutions use between 1500 and 3000 cGy [289, 290]. Considerations in selecting a dose include the ability of that dose to interfere with lymphocyte proliferation, the effect on the cells being given for therapeutic purposes, and the clinical experience with blood components subjected to the doses selected. A very low dose of radiation interferes with lymphocyte response to allogeneic cells. Gamma irradiation

at a dose of only 500 cGy will abolish lymphocyte proliferation in MLC [290, 291]. Irradiation with 1500 to 5000 cGy reduces the incorporation of ^{14}C-thymidine into mitogen-stimulated lymphocytes by 85–98.5% [292]. Doses of up to 5000 cGy do not have an adverse effect on red cells, platelets, or granulocytes [292, 293]. Red cell survival in vivo and in certain in vitro assays are normal after irradiation with up to 10,000 cGy [293]. Granulocyte chemotaxis may be slightly reduced by even 500 cGy, but this does not become significant until irradiation with greater than 10,000 cGy [291]. Very high doses such as 40,000 cGy are required to interfere with phagocytosis and microbial killing [291, 293, 294]. In vitro platelet function studies have generally been normal following irradiation with up to 5000 rads [292, 293]. However, at this dose there is a 33% reduction in in vivo recovery and diminished correction of the bleeding time by irradiated platelets [293], although since Button et al. [293] did not observe a difference in the platelet increment following transfusion of irradiated compared with nonirradiated platelets, they concluded that 5000 cGy was a clinically acceptable dose. Studies involving lower doses of irradiation such as 2500 or 3000 cGy showed normal in vivo survival [295, 296] and posttransfusion increments [297]. Thus, it appears that at the doses of radiation generally in use there is no interference with platelet function or survival.

One difficulty in selecting a radiation dose is the lack of a definitive in vitro assay to establish a clinically effective dose. The dose of radiation to prevent transfusion-associated GVHD was originally selected using the MLC assay. During the past few years, the limiting dilution assay (LDA) has been proposed as a better indicator of the effects of irradiation on lymphocytes because the LDA detects a reduction in viable T cells of 5 $\log_{10}$, compared with a reduction of 1–2 $\log_{10}$ detectable by the MLC assay [286, 298]. Studies using the LDA showed that a radiation dose of 2500 cGy completely eliminated T-lymphocyte growth [299], and, based on this experience, the US FDA requires a minimum dose of 2500 cGy [300].

Storage of irradiated components

In almost all of the studies on irradiation of blood components the cells were studied shortly after irradiation. As the use of irradiated blood components has increased, interest has developed in irradiating the components after collection and storing them for use days or weeks later. Red cells have reduced but acceptable in vivo survival when stored for 42 days following irradiation [301]. However, doses of 2000 or 3000 cGy to units of red cells result in potassium levels two and three times normal after storage for 4 to 5 days [302, 303]. This suggests that leakage of potassium from the red cells occurred during postirradiation storage, perhaps resulting from irradiation damage to the red cell membrane or the sodium–potassium pump. There has been some interest in washing irradiated red cells that have been stored for several days; however, in a thorough review of the situation Strauss concluded that washing is not

necessary for most clinical situations [304]. Based on these studies, red cells can be stored for only 28 days after irradiation.

Because there is no reduction in platelet recovery and survival when previously irradiated platelets are stored [296], platelets can be irradiated and stored for the normal 5 days. Red cells that have been irradiated can be frozen, stored, and thawed with no reduction in in vivo red cell recovery [305]. The effect of irradiating frozen red cells is not known, and although some authors [285] believe it is acceptable to irradiate frozen components, it seems more advisable to irradiate these components either before freezing or after deglycerolization.

Quality control of irradiation

Quality control of blood irradiators is extremely important to ensure that the components receive the expected dose. Moroff et al. [285] have proposed the following as appropriate quality control measures for blood irradiation:

1. Use of qualitative indicators to confirm that irradiation was performed as intended.
2. Periodic measurement over the delivered dose using appropriate dosimetric techniques.
3. Periodic surveys to detect isotope leakage.
4. Daily confirmation of timer accuracy.

Leukocyte depletion to prevent GVHD

Because blood filters are very effective in removing leukocytes, the question has arisen as to whether blood filtration might be an alternative to irradiation for prevention of GVHD. Present blood filters remove up to 99.9% of the leukocytes from red cells, platelets, and plasma, resulting in fewer than 5×10^6 leukocytes per unit [306, 307]. However, differences in patient size and in the degree of immunocompetence make it impossible to predict a minimum GVHD-producing dose of leukocytes for each patient. Because irradiation is almost completely effective in preventing transfusion-associated GVHD, there is no motivation to develop alternative GVHD prevention methods and almost no willingness by physicians to undertake clinical trials. This is particularly true since, although on average, filters produce components that contain fewer than 5×10^6 leukocytes, a small percentage of units contain considerably more leukocytes [308]. Thus, filtration is not an acceptable approach to prevention of transfusion-associated GVHD.

Because it is difficult to quantitate cellular immunity, there are no in vitro assays that define the degree of immunodeficiency that makes a patient susceptible to transfusion-associated GVHD. Even if better assays were available, these probably would not be helpful because the degree of HLA matching between the patient and blood donor may be a factor in the likelihood of transfusion-associated GVHD. In the situations described below, patients are so severely immunocompromised that transfusion-associated GVHD is very likely unless blood components are irradiated.

Pathogen inactivation and prevention of GVHD

Because pathogen inactivation technologies damage nucleic acids and prevent microbial duplication (see Chapter 5), this process also affects lymphocytes [227, 228, 286]. Thus, platelets subjected to pathogen inactivation will not cause GVHD and irradiation is not necessary. If /when pathogen activation is available for all blood components, it is possible that irradiation can be eliminated, but in the meantime pathogen inactivated components need not be irradiated [309, 310].

Indications for irradiated components
fetus

Fatal GVHD can occur from viable lymphocytes in blood used for intrauterine or exchange transfusion [311, 312]. All blood components used for these indications should be irradiated. Components transfused to these patients after delivery should also be irradiated.

Neonates

Although neonates do not have adult levels of immune competence, there are very few reports of transfusion-associated GVHD in neonates (see Chapter 12). There is no evidence that newborns who do not have a congenital immunodeficiency are at increased risk of developing transfusion-associated GVHD. Routine irradiation of blood components for all neonates is not recommended [313]. However, it has been proposed that infants whose birth weight is less than 1250 g should receive irradiated components because of their underdeveloped immune system [314].

Congenital immune deficiency

Patients with severe combined immunodeficiency syndrome or Wiskott–Aldrich syndrome have severe defects in immunity. These patients have a very high likelihood of developing transfusion-associated GVHD, even from one unit of fresh plasma, and should receive irradiated blood components [268, 269]. Patients suspected of having congenital immune deficiency should receive irradiated components until the diagnosis is refuted. DiGeorge syndrome involves immune deficiency due to thymic dysfunction. These patients should receive irradiated blood components [315].

Allogeneic bone marrow transplantation

These patients are severely immunocompromised by the irradiation with or without chemotherapy given to prepare them for the transplant and in many situations to eradicate residual disease. The GVHD that commonly follows allogeneic bone marrow transplantation is caused by viable lymphocytes in the donor marrow. Irradiation of all blood components has become routine; however, as a result there are no data describing the risk of development of transfusion-associated GVHD in allogeneic bone marrow transplant recipients. It can be presumed that the risk would be extremely high, and so irradiated blood components are used routinely. Unless the

patient has a disease that would necessitate the use of irradiated blood products, these are not necessary for stem cell transplant patients until the transplant preparative regimen is begun. Prior to that time, the blood components should be selected based on the patient's underlying disease.

Autologous bone marrow transplantation

Although there may be some differences in the irradiation and chemotherapy preparation for autologous compared with allogeneic bone marrow transplantation, patients undergoing autologous bone marrow transplantation are severely immunocompromised. Because the use of irradiated blood components was an established practice in allogeneic bone marrow transplantation, it has been adopted for autologous bone marrow transplantation without clinical or laboratory study.

Even though nonmyeloablative preparative regimens are being used increasingly, irradiation of blood components is recommended for these patients.

If transfusions are provided before or during the stage of peripheral blood stem cell collection, irradiation avoids the potential that viable donor lymphocytes could be ultimately transfused to the patient with the stem cell transplant. Although no such cases have been reported, irradiation avoids this theoretical situation [316].

Hematologic malignancies

Some patients with acute leukemia, lymphoma, or Hodgkin's disease who are receiving chemotherapy, often along with radiation, have developed transfusion-associated GVHD [274–276, 317–319]. The risk of transfusion-associated GVHD in these patients has been estimated to be between 0.1% and 1.0% [320], but this risk is difficult to determine because of the lack of comprehensive follow-up studies. The data currently available are insufficient to establish whether blood components for these patients should be routinely irradiated, but this is not usually done.

Aplastic anemia

These patients usually do not have defective cellular immunity. There have not been documented cases of transfusion-associated GVHD due to transfusion of normal cells. Thus, the use of irradiated blood components is not necessary. However, if these patients are undergoing very intensive chemotherapy regimens, it may be appropriate to irradiate blood components.

Solid tumors

One patient with neuroblastoma and one with glioblastoma have developed transfusion-associated GVHD [277, 317]. Some treatment protocols for these patients result in rather severe immune system compromise; however, the data are not adequate to recommend irradiation of blood products for these patients except in therapy involving severe immune deficiency.

Acquired immune deficiency syndrome

Despite the fact that these patients have severely impaired immune function, no cases of transfusion-associated GVHD have been reported. This may be because these patients receive irradiated blood components in some centers. However, the risk for AIDS patients of developing transfusion-associated GVHD is not known.

Granulocyte transfusions

During the 1970s, there was concern that granulocyte transfusion posed a greater risk of transfusion-associated GVHD than other cellular blood components [319]. However, this was probably due to the increased risk of transfusion-associated GVHD from transfused chronic myelogenous leukemia cells, not normal donor cells. Thus, there is no need to routinely irradiate granulocyte concentrates obtained from normal donors. Chronic myelogenous leukemia cells are no longer used for transfusions, and so that indication for irradiating granulocytes no longer exists. The decision to irradiate granulocytes should be based on the patient's underlying condition, not the cellular product.

Noncellular blood components

Transfusion-associated GVHD has occurred in patients with congenital immune deficiency following transfusion of fresh liquid plasma [267–269] but has not been reported to be caused by previously frozen components, FFP, or cryoprecipitate. These components contain fragments of leukocytes but few, if any, viable lymphocytes. They would not be expected to cause transfusion-associated GVHD. Although irradiation of FFP and cryoprecipitate is probably not necessary, many blood banks do irradiate these components to avoid clerical errors in which a cellular blood component might not be irradiated when necessary.

Components from partially HLA-matched, related, or unrelated donors

Several years ago, some cases of transfusion-associated GVHD occurred after transfusion from relatives who were partially HLA-matched with the patient or from unrelated but partially HLA-matched donors [268, 269]. This raised the theoretical concern that transfusion-associated GVHD might develop in patients not severely immunocompromised if there was partial HLA matching between the patient and the blood donor. However, since most of the patients involved in these reports were severely immunocompromised, this remained a theoretical concern until 1989. Then several reports appeared suggesting this situation might be more common than previously believed. Thaler et al. [278] reported transfusion-associated GVHD apparently caused by fresh blood from children in two immunocompetent patients who underwent cardiac surgery. In each case one of the blood donors was homozygous for an HLA class I antigen haplotype shared with the recipient. Thus, the recipient would not have recognized the HLA class I antigens as foreign. There have now been additional reports of transfusion-associated GVHD in Japan in

patients who received fresh blood after cardiac surgery, in a woman who was transfused following delivery [279], in a cardiac surgery patient in New York [280], and in a Japanese woman transfused after cholecystectomy [281]. The apparent high incidence of this situation in Japan may be due to the rather high frequency of certain HLA antigens in the Japanese, with the resulting likelihood that a random unrelated donor may be partially HLA-matched with the recipient (see Chapter 16). The problem can be prevented by irradiating the blood components donated by first-degree relatives of the patient and this is now required (321).

If the component, usually platelets, is selected because it is an HLA match with the recipient, there is a possibility that it will not demonstrate HLA antigens that the recipient lacks and, thus, would not be rejected by the recipient. However, the recipient might possess HLA antigens that the donor lacks, creating a donor–recipient mismatch similar to that described above in related donors. The incidence of transfusion-associated GVHD in immunocompetent patients in the United States is not known. There is no evidence that this is a problem when unrelated donors are used; however, the use of directed donors who are related to the patient may increase the likelihood of transfusion-associated GVHD. Thus, all HLA-matched components should be irradiated. Irradiation is also required for components selected for HLA compatibility by crossmatching. Although the platelet crossmatch is not a specific HLA test, it is presumed that this does select donors who are more likely to be HLA matched with the recipient and, thus, have the potential to cause transfusion-associated GVHD.

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12 Transfusion Therapy in Specific Clinical Situations

12.1 Acute blood loss

Physiology and therapy

The signs, symptoms, and physiologic changes that occur in association with different degrees of blood loss are rather well known. Loss of approximately 10% of the blood volume causes few symptoms (Table 12.1) and this is what happens thousands of times each day when people donate blood for transfusion.

When patients experience acute blood loss, the primary need is for volume replacement. This need is more urgent when the extent of the blood loss is greater. Initially, blood loss depletes the intravascular space, and only later is there a shift of fluid from the extravascular into the intravascular space. Thus, early in the blood loss situation, attention can be focused on replacing intravascular space losses, and this can be accomplished easily using crystalloid solutions such as isotonic saline or Ringer's lactate [1]. As blood loss continues, fluid shifts from the extravascular space to compensate for the decreased intravascular volume. Because crystalloid solutions are distributed into the extravascular space as well, it is necessary to administer two to three times the amount of crystalloid as the volume lost. Thus, if large volumes of crystalloids are given, there is a risk of fluid overload, especially in the elderly. There has been a debate on whether the use of colloid solutions is preferable, since these solutions would maintain the intravascular oncotic pressure without providing the large amount of extra fluid [2]. The solutions are compared in Table 12.2. The results of clinical studies have not resolved this issue [3–5]. Crystalloid solutions are recommended as the initial treatment of acute blood loss. If blood loss continues and represents a substantial portion of the blood volume, colloid solutions can be added because crystalloid solutions leave the intravascular space rather rapidly. The colloid solutions used are 5% human serum albumin, hydroxyethyl starch (HES), or 5% plasma protein fraction (see Chapters 2 and 5). These products provide replacement of the volume lost on an equal basis. HES may actually provide a slightly larger volume replacement than the volume administered, although some physicians prefer not to use it because of its effect on coagulation.

Table 12.1 Reaction to acute blood loss of increasing severity.

% TBV (total blood volume)	Blood loss (mL)	Clinical signs
10	500	None. Vasovagal syncope in blood donors is rarely seen.
20	1000	Impossible to detect volume loss. When the patient at rest tachycardia is usual with exercise, and a slight postural drop in blood pressure may be evident.
30	1500	Neck veins are flat when supine. Postural hypotension and exercise tachycardia are generally present, but the resting, supine blood pressure and pulse still can be normal.
40	2000	Central venous pressure, cardiac output, and arterial blood pressure are below normal even when the patient is supine and at rest. The patient usually demonstrates air hunger; a rapid, thready pulse; and cold, clammy skin.
50	2500	Severe shock, death.

Volume lost up to — column header spanning % TBV and Blood loss.

Source: Hillman RS. Acute blood loss anemia. In: Beutler E, Lichtman MA, Coller BS, et al., eds. Williams Hematology, 5th ed. New York: McGraw Hill, 1995:704–708.

   The symptoms that occur with blood loss are the result of blood volume depletion, not depletion of red cell mass. Blood flow to and oxygenation of the brain and heart are maintained by adrenergic nervous system stimulation, release of vasoactive substance, hyperventilation, shift of fluid from intracellular to extracellular space, shift of fluid from the interstitial to the intravascular space, and renal conversion of water and electrolytes. Two major points deserve emphasis: (a) because the manifestations of acute blood loss are due to hypovolemia, early and aggressive replacement of intravascular volume is essential; and (b) unless the patient has a very low initial hemoglobin concentration or has severely impaired cardiovascular function, red cell replacement is not necessary during initial

Table 12.2 Comparison of crystalloid and colloid solutions.

	Crystalloid	Colloid
Intravascular retention	Poor	Good
Peripheral edema	Common	Possible
Pulmonary edema	Possible	Possible
Easily excreted	Yes	No
Allergic reactions	Absent	Rare
Cost	Inexpensive	Expensive

Source: Stehling L. Fluid replacement in massive transfusion. In: Jefferies LC, Brecher ME, eds. Massive Transfusion. Bethesda, MD: American Association of Blood Banks, 1994:1–16.

therapy of acute blood loss. Initial resuscitation with blood is also not practical because of logistic difficulties. With the shift to blood component therapy, whole blood is rarely available, and it is impractical to maintain an adequate stock of "universal donor" group O negative red cells for every situation. Experience with conservative use of red cells (see Chapter 11) and bloodless medicine [6–9] also illustrates the variety of ways patients can be managed without the use of red cells. Thus, the initial management of acute blood loss is as described here. If blood loss continues, the situation becomes one of massive transfusion and then there are additional therapeutic considerations.

Blood bank procedures

The treatment of acute blood loss usually occurs in an unexpected emergency situation. Because of this stress and urgency, errors can occur, and it is extremely important that strict procedures be followed for requesting and distributing blood and identifying the patient. Effective communication between the physicians or key personnel on the patient care team and the blood bank is essential. Blood bank personnel are well aware that there may be situations in which there is an urgent need for blood, and each blood bank should have a procedure for the rapid release of red cells. Busy emergency departments usually have well-defined plans for these situations; however, when acute blood loss occurs in other situations, clinical care personnel may be less familiar with the policies and procedures necessary for rapid, safe provision of blood components. In these situations, clear, concise, and informative communication between patients, the physician, and the blood bank and the blood bank to the physician is essential. To avoid frivolous release of incompletely crossmatched or noncrossmatched red cells, the blood bank personnel will usually ask questions about the patient's situation. It is essential that a physician take responsibility for the situation and clearly indicate the urgent nature of the patient's situation to the blood bank personnel. After the emergency has passed, the physician will be expected to sign a form taking responsibility for the emergency release of the blood. The most important feature of the communication is for the clinical care team to provide the blood bank with a clear, concise assessment of the situation and the anticipated blood needs. Initially it may be difficult to predict the needs, and so continuing communication is important. The blood bank has a variety of ways to respond, depending on the patient's needs (see Chapter 10). Many blood banks have established massive transfusion protocols to deal with this situation (it is discussed later in the chapter).

First, a blood specimen should be obtained and sent to the blood bank for emergency type and crossmatch. An ABO and Rh type can be performed quickly and blood of the same type as the patient can be selected. This blood can be released under an emergency crossmatch procedure, which requires approximately 15 minutes (see Chapter 10). Thus, partially crossmatched blood of the patient's type can be available quickly. Blood released using this emergency crossmatch procedure involves shortening the incubation of the patient's serum and the donor's

red cells. Usually this is done in a way to detect only ABO incompatibility, since that is usually the most disastrous kind of transfusion reaction. In the past, blood was often released without any crossmatch. Today, most blood banks have simplified procedures to carry out a rapid crossmatch that will detect ABO incompatibility in a very few minutes to avoid compromising patient care. If it is considered necessary to release blood without a crossmatch and if the blood bank has a sample of the patient's blood, the ABO type can be determined quickly and ABO type-specific blood released without a crossmatch. Usually Rh-positive blood would be chosen, since 85% of patients will be Rh-positive and the inventory of Rh-negative blood is limited. In some situations, Rh-negative blood may be used depending on the hospital's inventory and the age and sex of the patient. When the patient's ABO type is not known, group O red cells are used. This has led to the designation of group O Rh-negative individuals as a universal donor, since these red cells would not be hemolyzed by either anti-A, anti-B, or any Rh(D) antibody present in the patient and would not immunize patients to the D antigen. Group O Rh-negative (universal donor) red cells do not avoid the potential risk that the recipient may have another red cell antibody or a red cell autoantibody, and so hemolysis or transfusion reactions can occur following transfusion of group O Rh-negative "universal donor" red cells. Stocking O Rh-negative red cells routinely in emergency departments is neither necessary, practical, nor appropriate. The red cells may not be stored properly, and there may not be a system of checks for release of the units—practices that can lead to serious errors. In addition, there is not an adequate national supply of O negative red cells to provide these for every emergency department. Techniques of fluid management and resuscitation are so highly developed today that patients can be maintained for the very few minutes required to obtain red cells from the blood bank.

Changing blood types

During acute blood loss or massive transfusion, the supply of the patient's specific blood type in the hospital may not be sufficient to meet the need. Thus, it may be necessary to change blood types. Several factors should be considered (Table 12.3). Patients with less common types of AB or B can be switched to the more common type A (from AB) or O (from B). This switch means that patients will be receiving plasma containing

Table 12.3 Factors to consider in changing blood types in acutely bleeding patients.

The patient's immediate clinical condition
The patient's overall diagnosis
The patient's blood type
The hospital's blood inventory
Responsiveness of the blood supplier
The supplier's blood inventory

ABO-incompatible antibodies. Although most of the plasma is removed in the production of red blood cells, transfusion of large numbers of ABO-incompatible units can provide sufficient antibodies to cause a positive direct antiglobulin test (DAT) and/or hemolysis. Appropriate monitoring of these patients involves testing for ABO antibody, performing a DAT, and observing the patient for a decrease in hemoglobin not attributable to other causes.

For Rh-negative patients, it may be necessary to convert to Rh-positive blood. If these patients also have the less common ABO types (AB or B), converting only the Rh type but continuing to use the patient's own ABO type may not provide much additional blood. Thus, if the clinical situation indicates a continuing need for a large amount of blood, it is usually advisable to switch AB or B negative patients to A or O positive (respectively) red cells. For A or O negative patients, the only option is to switch to A or O positive red cells. If the supply of Rh-negative red cells is inadequate to meet the patient's needs, there should be no hesitancy in switching to Rh-positive red cells. It is most important to deal with the patient's immediate transfusion needs and address the possibility of Rh immunization later. In women of childbearing age, Rh immune globulin can be used to prevent immunization (see Chapter 10).

Once the patient is stabilized, bleeding is controlled, and blood inventory is adequate, consideration can be given to switching the patient back to his or her original type. The major criterion for this switch is the absence of circulating ABO antibody incompatible with the patient's original type. As long as ABO incompatible antibody remains, the crossmatch should be incompatible. The patient's serum should be tested in the antiglobulin phase or by an equally sensitive method to ensure the absence of free ABO-incompatible antibody. When the crossmatch becomes compatible, the patient can be switched back to the original ABO type. There is a theoretical concern that in the absence of free ABO antibody, antibody bound to the patient's red cells could re-equilibrate and coat the newly transfused cells, thereby reducing their survival. It is not known whether this occurs, and the presence or absence of free antibody is a suitable indicator of safety.

Massive transfusion

Massive transfusion may be defined in several ways (Table 12.4). Many different clinical situations may lead to massive transfusion, and the indications for transfusion are as diverse as the clinical situations [10]. The potential complications of massive transfusion are the results of the

Table 12.4 Definitions of massive transfusions.

Replacement of the patient's blood volume during a 24-hour interval
Transfusion of more than 20 units of red cells in 24 hours
Replacement of more than 50% of the patient's blood volume in 3 hours
Blood loss more than 150 mL/minute in an adult

Table 12.5 Changes in red blood cell units during storage.

Decreased pH
Increased affinity of hemoglobin for oxygen
Decreased red cell deformability
Hemolysis
Increased potassium concentration
Increased ammonia concentration
Increased phosphate concentration
Development of microaggregates
Deterioration of platelets
Decrease in factors V and VIII
Research of vasoactive substances
Denaturation of proteins

biochemical and functional characteristics of stored blood (Table 12.5). Most red cells are suspended in an additive solution to optimize the quality of cells and length of storage. This means that virtually all of the plasma and platelets have been removed, and thus in massive transfusion of red cells these other blood components are not replaced. These changes in stored red cells, as well as the citrate content and cold temperature, create potential for substantial complications (Table 12.6) when large volumes are transfused rapidly. The tread of hypothermia, acidosis, and coagulopathy have a very bad prognosis.

Ten to fifteen percent of red cell units are used in trauma. In one study of 5645 patients with an overall mortality of 27%, 479 received red cell transfusions and 62% of red cells were given in the first 24 hours of care. Three percent of patients received 71% of the red cells and the mortality in that cohort was 39%. There is no clear threshold beyond which continued transfusion was fatal [11]. Positive experience with providing combinations of red cells, fresh frozen plasma (FFP), and platelets quickly to patients experiencing massive acute blood loss/trauma has led many hospitals to establish massive transfusion protocols [12]. These protocols are clinically helpful in trauma, massive transfusion, and postpartum hemorrhage [13] and experience is refining the RBC : FFP : platelet ratios [14, 15].

Table 12.6 Potential complications of massive transfusion.

Thrombocytopenia
Coagulopathy
Hypothermia
Acidosis
Poor oxygen dissociation
Hypocalcemia
Hyperkalemia
Adult respiratory distress syndrome due to microaggregates
Hyperammonemia
Plasticizer toxicity

Coagulopathy

Well-defined coagulation disorders can be identified in one-half to two-thirds of patients receiving a massive transfusion [16–20] and after 12 units of red cells in a short time, most patients have a coagulopathy [17], although this does not necessarily mean that such patients bleed abnormally [21, 22]. In the past, the focus on the first six hours of acute hemorrhage involved utilization of crystalloids or occasionally colloids, but there was very little focus on the coagulopathy that developed during that time. The strategy was based on research using controlled hemorrhage and lactated ringers while getting blood ready, but this evolved into the use of large amounts of lactated ringers.

Thrombocytopenia is the most common coagulation abnormality in patients receiving a massive transfusion [17, 18]. The platelet count is usually inversely related to the number of units of blood transfused [17]. Transfusion of large amounts of blood depleted of platelets and coagulation factors may create deficiencies in the recipient because of dilution of the recipient's blood with this depleted stored blood. In addition, the hemostatic process consumes the patient's own platelets and coagulation factors and adds further depletion. Usually factor VIII is rapidly replaced by the patient, and factor V levels do not fall below that needed for hemostasis. Measurement of fibrinogen, which is not depleted in stored blood, is also often helpful as an aid in diagnosing disseminated intravascular coagulopathy (DIC) [17, 18], but in general the prothrombin time (PT), partial thromboplastin time (PTT), and bleeding time are not helpful in elucidating the cause of abnormal bleeding [17, 19] because there is little relation between the level of coagulation factors and bleeding [17, 23].

In severely injured patients or situations of massive transfusion, a triad of hypothermia, acidosis, and coagulopathy develops and leads to very high mortality. Conventional practice has involved reversal of acidosis, prevention of hypothermia, and surgical control of hemorrhage. Early intervention to prevent or treat coagulopathy has generally not been used and instead the focus has been on replacing blood loss and maintaining blood volume. Military experiences have begun to support the importance of treating coagulopathy much earlier and the liberal use of crystalloids may actually worsen acidosis and coagulopathy [24].

An approach has been developed involving hypotensive management (BP < 90 mm/hg) to minimize continued bleeding and restoration of intravascular volume using thawed FFP in a one-to-one or one-to-two ratio with packed red blood cells (PRBCs). Civilian and military trauma centers have become increasingly sophisticated in dealing with severe injury and uncontrolled coagulopathic hemorrhage is now the major cause of preventable death following trauma [25]. Most such patients are coagulopathic on admission and this is associated with poor outcome [26]. Recognition that coagulopathy is present very soon after trauma has led to measures to prevent coagulopathy or treat it promptly. Most patients who present at trauma centers do not have coagulopathy but based on clinical evaluation or rapid point of care laboratory testing, those patients with coagulopathy or likely to develop it can be recognized early. For instance,

the severity of hypotension, acidosis, hypothermia, and injury severity can identify patients almost certainly coagulopathic. This has resulted in improved survival in the military setting [27, 28] with the use of standard "package" from the blood bank of red cells, plasma, and platelets in a 1 : 1 : 1 ratio [29]. This is a major change from past practices of proving these components separately and only after coagulopathy develops but appears to be effective [30–33] and is becoming widely used. A similar strategy involving three-to-two ratio of RBCs to FFP has been effective in the civilian trauma setting [14, 29, 34]. Fibrinogen concentrate is now available (Chapter 5) and may be of value in this situation [35].

In a busy general acute care hospital, patients experiencing acute blood loss may account for a substantial portion of blood use [36].

Activated factor VII in acute blood loss

In patients with multiple coagulation factor deficiencies as in massive transfusion, the factors can be replaced using FFP or hemostasis can be achieved by activating the external pathway with the use of activated Factor VII (Factor VIIa) [37]. Factor VIIa has been used prophylactically and therapeutically but a review of 17 clinical trials did not show a benefit [38] other than possibly early in the management of intracranial hemorrhage. A separate summary of 483 articles, including 28 clinical trials, also did not reveal definitive evidence of benefit [39]. Thus, there is no body of evidence that Factor VIIa is of value in massive transfusion despite its rather widespread use and one summary of 35 clinical trials found an increased risk of arterial thrombosis in patients receiving VIIa [40].

Hemoglobin function

2,3-Diphospho-glycerate (DPG) controls the release of oxygen from hemoglobin [41, 42]. During blood bank storage, red cell DPG levels decline [43] (see Chapter 5). This is associated with a shift in the oxygen dissociation curve of hemoglobin to increase oxygen binding and decrease the ability to release oxygen to the tissues [44]. Although DPG levels are regenerated and oxygen dissociation returns to nearly normal about 24 hours after transfusion [45], there has been concern that massive transfusion of DPG-depleted red cells might result in poor oxygen delivery. The clinical importance of this has been difficult to determine [46]. Studies have shown that animals can compensate very well for DPG-depleted red cells if the hematocrit and blood volume are maintained [47, 48]. In humans, cardiac output and oxygen extraction are far more important than the small contribution to oxygenation made by increased levels of DPG. Thus, it appears that in most humans moderate depletion of DPG is tolerated [49]. In patients with chronic anemia or compromised cardiopulmonary function, low DPG levels may be detrimental, but there is little evidence supporting this.

Hypocalcemia

The likelihood of development of hypocalcemia due to the infusion of large amounts of citrate during massive transfusion has been

overemphasized [50]. Routine administration of calcium during massive transfusion is probably not necessary. Up to one unit of red cells can be administered every 5 minutes to adults with normal body temperature and who are not in shock [51]. Of primary concern regarding hypocalcemia are its cardiovascular effects, which occur before hypocalcemia is severe enough to cause coagulopathy. The effects of rapid citrate infusion are described in more detail in Chapter 7.

Hypothermia

If large amounts of cold blood are transfused rapidly, hypothermia may result. Cardiac arrhythmia may occur, oxygen and energy requirements are increased, metabolism of citrate and lactate are impaired, potassium is released from the intracellular space, and the affinity of hemoglobin for oxygen is increased [52]. Thus, in massive transfusion, warming of blood to approximately 37°C is advisable (see Chapter 13).

Acid–base balance

Stored blood contains an acid load, primarily due to citric acid and lactic acid. This may be an exaggerated problem because patients undergoing massive transfusion may have a metabolic acidosis. However, worsening acidosis is often related to the inability to control hemorrhage and shock. Routine administration of alkalinizing agents in these patients gives an additional sodium load and might shift the oxygen dissociation curve to impair the release of oxygen from red cells. Thus, use of alkalinizing agents should be based on specific results of monitoring the patient rather than predetermined arbitrary schedules.

Microaggregates

The standard blood filters have a pore size between 170 and 230 mm. During the 1970s, it was recognized that microaggregates of 20 to 120 mm composed of platelets, leukocytes, and fibrin strands develop in stored blood [53]. It was believed that these were important causative factors in the development of adult respiratory distress syndrome (ARDS), and thus microaggregate filters were developed. As the pathophysiology of ARDS was better understood, it became clear that this is a complex situation and that microaggregates are not the primary cause. Microaggregate filters are available and are used in some centers, but they do not achieve substantial leukocyte reduction; the increasing use of leukodepleted components is eliminating the need for microaggregate filters.

Plasticizers

Plasticizers from the bags accumulate in red cell components during storage [54] and can be found in tissues of multitransfused patients [55, 56]. However, there is no evidence that transfusion of this material causes clinical problems [57].

Electrolytes

Potassium, ammonia, and phosphate levels are elevated in stored blood, but this usually does not cause clinical problems [58]. Potassium levels can

become quite high in stored red cells (see Chapter 5), but the total amount of potassium is not large because of the small volume of additive solution in which the red cells are suspended. Therefore, in most situations, this dose of potassium is not clinically dangerous. There are case reports of cardiac arrhythmia or fatality apparently due to hyperkalemia [59–61], but the complexity of the clinical situation makes it difficult to establish clear cause-and-effect relationships and specific transfusion guidelines. It is important to be aware that relatively small amounts of blood can be a massive transfusion for small patients.

Blood samples for laboratory tests

An issue that sometimes arises is the effect of massive transfusion on the validity of laboratory test results. There are no applicable standards or published guidelines. The effect of transfusion on an analyte will depend on the (a) analyte, (b) blood component, (c) age of the blood component, (d) volume of transfusion related to the size of the patient, and (e) patient characteristics [62]. There are very few reports of transfusion interfering with test results and this does not seem to be a large problem. It is advisable not to draw blood samples for laboratory testing during or for 1 hour after a transfusion and not from the line being used for transfusion. If it is necessary to obtain the sample during a transfusion, this should be noted so it can be taken into account when the test results are interpreted.

12.2 Cardiovascular surgery

Techniques for preventing perioperative myocardial ischemia are generally effective [63]. Decisions to transfuse red cells are based on the patient's general cardiovascular health and the amount of blood loss. Patients undergoing cardiopulmonary bypass often develop thrombocytopenia, platelet function abnormalities, and abnormal blood coagulation due to depletion of factors V, VII, VIII, and IX [64–69]. The coagulation and platelet abnormalities are thought to be caused by hemodilution, activation of platelets by the cardiopulmonary bypass instruments, fibrinolysis, inadequate neutralization of heparin, and alteration of von Willebrand factor [65]. However, most patients do not experience unusual bleeding and the extent of bleeding is not associated with these hemostatic abnormalities, but is usually impaired surgical hemostasis [64–68]. Thus, the routine transfusion of plasma components or platelets following cardio-pulmonary bypass is not indicated [67]. Although there is considerable variability in transfusion practices in these patients [70], patients who develop excessive bleeding should be managed like any other surgical patients; that is, transfusion is indicated if the platelet count is less than 50,000/mL or if a severe platelet function defect is present [71]. The problem is that it can be presumed that all patients will have some degree of platelet dysfunction following cardiopulmonary bypass, so it is essential to determine whether excessive bleeding is caused by platelet dysfunction or anatomic problems. Platelet transfusion in patients undergoing

coronary artery bypass graft surgery was associated with increased risk of serious adverse events in retrospective analysis of double blind placebo controlled Phase III trials for licensure of aprotinin [72]. It is not clear whether platelet transfusion has a causal role in these adverse events or is an indication of sicker patients [72, 73].

The routine use of FFP or cryoprecipitate is not necessary, although the use of desmopressin acetate may reduce blood loss in patients undergoing complex cardiovascular procedures [74]. The thromboelastogram has been suggested as helpful to identify bleeding due to coagulopathy in these patients, but it is not clear that the results correlate well with the clinical situation. Because the thromboelastogram measures both platelet function and fibrin formation, it is not helpful in selecting the appropriate component [75].

The PFA-100 is a point-of-care assay that evaluates platelet reactivity by measuring the closure time of a membrane opening. Various platelet aggregate agents are used to stimulate platelet activity. This has been advocated as a method to determine the likelihood of bleeding in aspirin-treated patients or other patients with possible platelet function defects. The device is not diagnostic or specific to any particular platelet function disorder. While some experience has been promising [76], this device has not gained widespread use and further carefully controlled studies are necessary in order to determine its long-term value.

The volume of blood lost by patients undergoing cardiopulmonary bypass is now relatively small, usually one to six units [9, 70]. Predicators of transfusion include: previous heart surgery, platelet count, baseline hemoglobin, and ejection fraction [68]. Blood can be replaced using routine red cell components according to usual blood bank practices. Because the red cells may undergo some trauma in the bypass instrument, it is advisable that not all units of red cells be near the end of their storage period.

Some advocate the use of fresh blood (< 48 hours) for pediatric patients, but other studies – have not found a benefit and fresh blood is not usually used.

Stored RBC's in cardiovascular surgery patients

Most blood banks use a first-in-first-out inventory management system, and thus, the oldest red cells go to the patients most likely to be transfused such as cardiovascular surgery patients. The changes in red cells during storage are not well understood, but some studies suggest that longer stored red cells may be detrimental in several situations including cardiovascular surgery (reviewed in Lelubre C, Piagnerelli M, Vincent JL, 2009 [77]). One particular study [78] showing increased mortality in cardiovascular surgery patients who received longer stored red cells received considerable attention. However, the study had several shortcomings and the results have not been widely accepted.

LeLubre et al. [77]have nicely summarized the clinical trials of age of RBCs in four situations (see Chapter 5). Eight of those trials were in cardiovascular surgery patients. Four showed some effect and four did not.

All studies had substantial shortcomings such as being retrospective, small, single center, using different endpoints, and different ways of designating the age of all cells transfused. The authors conclude that available data "does not support a clinically relevant relationship" between red cell age and patient outcome [77, 79].

Autologous blood in cardiac surgery

This is discussed in Chapter 6.

12.3 Hematopoietic cell transplantation

The blood bank has an important role in a hematopoietic cell transplantation (HCT) program [80, 81]. These patients have unique transfusion requirements, use considerable amounts of blood components, and place a substantial demand on the blood bank [80–82] (Table 12.7), although autologous transplant can be performed without blood use [83]. There is a need to minimize the likelihood of alloimmunization, the severe immunosuppression these patients undergo, their temporary inability to produce blood cells, and the fact that their blood type may change and they may become a temporary or permanent chimera. Since transfusion therapy may differ before and after transplant, these situations will be considered separately.

Pretransplant

Before transplant, HCT patients are not immunosuppressed and thus may become alloimmunized to leukocytes or platelets following transfusion. This alloimmunization may cause transfusion reactions but, more importantly, may interfere with engraftment or with platelet transfusions (Chapter 11) needed during the transplant period. The presence of HLA antibodies is associated with marrow graft rejection [84] and pretransplant transfusions are associated with decreased patient or graft survival [85]. Formation of neutrophil antibodies results in delayed engraftment [86, 87]. Leukocyte depletion is effective in reducing the likelihood of alloimmunization following transfusion (see Chapters 11 and 14) and so patients should receive leukodepleted blood products in the pretransplant phase.

The indications for transfusion of the various blood components are the same as indications for other relatively stable anemic, thrombocytopenic, or leukopenic patients [81]. Because of the potentially serious adverse effect of transfusion on marrow engraftment and survival in these patients, transfusion should be given only after careful consideration of the patient's condition. For patients who require red cell or platelet transfusions before transplantation, the following is recommended:

1. Red cells should be depleted of leukocytes.
2. Platelet concentrates should be depleted of leukocytes.
3. The use of single-donor instead of pooled random-donor platelet concentrates should be considered. It has been thought that use of single-donor

Table 12.7 Blood component utilization by patients undergoing bone marrow transplantation at the University of Minnesota.

	Related				Unrelated				Autologous			
	% Transfused	Total used	Units range	Units median	% Transfused	Total used	Units range	Units median	% Transfused	Total used	Units range	Units median
Red blood cells	98	1077	0–75	18	100	856	6–93	15	100	1124	4–95	14
Peds red blood cells	1	1	0–1	0	13	3	0–1	0	8	7	0–3	0
Fresh frozen plasma	30	311	0–47	0	51	415	0–115	2	15	628	0–167	0
Peds fresh frozen plasma	9	4	0–1	0	16	5	0–1	0	5	4	0–2	0
Cryoprecipitate	9	115	0–36	0	16	104	0–24	0	9	256	0–184	0
Random platelet	89	2800	0–235	36	76	2311	0–977	17	74	1757	0–188	14
Apheresis platelet	98	2017	0–115	24	100	1460	4–172	32	100	1740	3–77	21
Apheresis platelet (split)	54	70	0–8	1	27	27	0–7	0	17	12	0–2	0
HLA platelet	26	118	0–37	0	35	213	0–41	0	22	136	0–30	0
Granulocytes	7	34	0–8	0	16	147	0–52	0	6	46	0–10	0
Granulocyte platelets	5	42	0–27	0	16	54	0–31	0	1	16	0–16	0
CMV-negative product	37	2248	0–277	0	19	1969	0–1258	0	38	1342	0–219	0
Leukocyte removal	40	1673	0–342	0	30	1703	0–1168	0	31	1462	0–351	0

Source: McCullough J. Collection and use of stem cells; role of transfusion centers in bone marrow transplantation. Vox Sang 1994; 67(Suppl 3):35–42.

platelets might delay alloimmunization by limiting the number of donor exposures. However, the largest, most recently completed study did not show a difference in the rate of alloimmunization between single-donor and pooled random-donor platelet concentrates [88].

4. Family members should not be used as blood or component donors (before transplant) because of the risk of alloimmunization and an unsuccessful marrow graft [89].

5. Cytomegalovirus (CMV) negative patients should receive CMV safe blood components.

Patients with inborn errors of metabolism or other nonmalignant diseases rarely require transfusion before HCT. If transfusion is necessary, these patients should be managed similarly to patients with aplastic anemia. Patients with malignancies who will undergo HCT usually have received multiple transfusions during the initial chemotherapy of their underlying disease. However, the effects of this on subsequent marrow engraftment are not as severe as for aplastic anemia patients because the chemotherapy is extremely immunosuppressive. Thus, transfusion therapy for these patients can be that necessitated by their chemotherapy. However, transfusions from family members should be avoided [89]. These patients usually have no transfusion requirements in the immediate pretransplant period because they are in hematologic remission.

Posttransplantation

Because of the severe immunosuppression caused by the pretransplant preparative regimen, fatal graft-versus-host disease can occur due to transfusion of viable lymphocytes in blood components [90]. Transfusion-related, graft-versus-host disease can be prevented by irradiating all blood components with at least 2500 rads (see Chapter 11).

Different time gaps occur between transplantation and marrow engraftment as different sources of stem cells are used for transplantation. The return of production of different blood cell lines varies, so although the duration of transfusion therapy may range from 2 to 6 weeks, the need for different components varies with different types of transplants (Table 12.7). Almost all patients require platelet and red cell transfusions, but since marrow transplantation does not usually interfere with the production of coagulation factors, transfusions of FFP and cryoprecipitate are necessary only if complications such as DIC develop. Although marrow transplant patients are severely neutropenic, granulocyte transfusions are not usually necessary.

The indications for the transfusion of blood components are the same as those for other kinds of patients. Because of their complex situation, these patients may place a major demand on the blood bank for blood components, especially platelets (Table 12.7). Even though these patients are recovering from severe immunosuppression as part of the preparative regimen for the transplant, they can become alloimmunized after transplantation [80, 91]. Thus, transfusion practices must take this possibility into account. Leukodepleted red cells are used to minimize the likelihood of inducing alloimmunization. Another unique consideration in

the transfusion management of HCT patients is the source of platelets. Either random-donor or single-donor (apheresis) platelets can be used. Some authors have advocated the use of single-donor platelets to delay alloimmunization and platelet refractoriness [92, 93]; however, a definitive study showed no difference in the rate of alloimmunization between pooled random-donor and single-donor platelets [88]. The major benefit was leukodepletion to reduce alloimmunization [88, 94–99]. Thus, leukodepletion of all blood components has become the standard practice for marrow transplant patients.

If patients become refractory to platelet transfusion, HLA-matched platelets from an unrelated or family donor can be used. Many marrow transplant programs use family members as donors following the transplant because they may be partially HLA-matched and they are readily available and motivated to donate often. This and other approaches to the management of patients refractory to platelet transfusion are described in more detail in Chapter 11. Another unique feature of HCT patients is that the marrow donor is available as a potential platelet donor. Platelets are occasionally obtained from the marrow donor if the patient is refractory and experiencing serious bleeding problems, but this decision requires considerable thought because of ethical considerations regarding the donor and is rarely done.

For CMV negative patients, all cellular components should be CMV safe (see Chapters 5 and 11).

ABO- and Rh-incompatible transplants

ABO incompatibility between donor and recipient does not preclude marrow transplantation [100–105] because it appears that hematopoietic stem cells lack ABH antigens [101]. ABO-incompatible transplants may be done when there is patient antibody directed against donor cells (major incompatibility; i.e., patient O, donor A) or donor antibody directed against the patient's red cells (minor incompatibility; i.e., patient A, donor O). Each of these situations has unique potential complications that present challenges to the transfusion service [102] (Table 12.8). In general, blood components given near the time of HCT should be compatible with both the donor and the recipient to minimize the chance of red cell hemolysis. Therefore, it may be necessary to use components of different ABO types at different times during the patient's course (Fig. 12.1).

When ABO-incompatible marrow is to be transfused into a patient with circulating antibodies against the donor's ABO type (e.g., A marrow into O patient), hemolysis of the red cells in the marrow can be expected. Some years ago, plasma exchange was used to reduce the level of circulating antibody in the recipient, but this was of only temporary value and high titers of antibody returned quickly, leading to hemolysis [103]. This problem is now avoided by processing the marrow to remove the red cells (see Chapter 18). Following transplant and after engraftment, the patient will become type A but cannot begin to receive type A red cells until the original patient circulating anti-A has disappeared (Fig. 12.1). Anti-A persists longer than anti-B [104]. Thus, even after the transfusion of type A

Table 12.8 Theoretical immunohematologic consequences of ABO-incompatible bone marrow transplants.

Minor ABO incompatibility
Anticipated problems
  Graft-versus-host disease
  Immune hemolysis at the time of infusion of the donor marrow caused by red
  cell antibodies caused by the donor marrow
Unanticipated problem
  Immune hemolysis of delayed onset caused by red cell antibodies produced by
  the donor marrow

Major ABO incompatibility
Anticipated problems
  Failure of stem cell engraftment
  Delay in onset of hematopoiesis, especially erythropoiesis
  Acute hemolysis at the time of infusion of the donor marrow
  Delayed onset of hemolysis associated with persistence of anti-A and/or anti-B
  after transplantation
  Hemolysis of infused red cells of donor type
  Hemolysis of red cells produced by the engrafted marrow
Unanticipated problem
  Mixed hematopoietic chimerism

Source: Petz LD. Immunohematologic problems associated with bone marrow transplantation. Transfus Med Rev 1987;1:86.

marrow, the patient continues to receive type O red cells containing additional anti-A that can slow the appearance of type A red cells. Usually, this does not lead to active hemolysis. If red cell engraftment is unexpectedly delayed, this is more likely the result of persistence of the patient's original antibodies than the transfusion of additional antibodies.
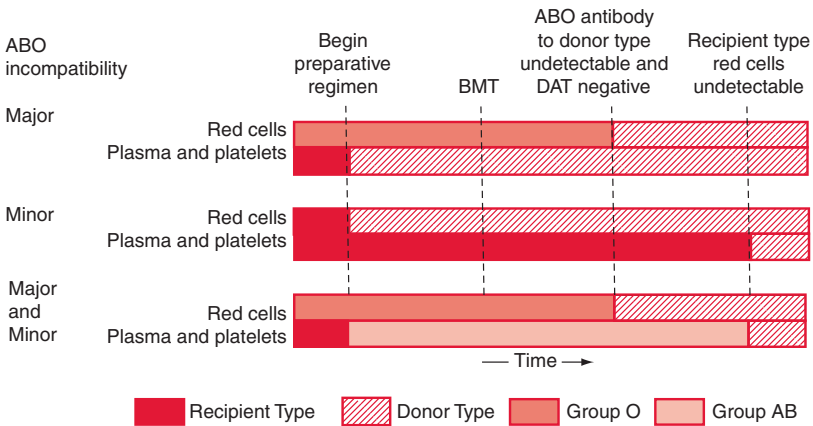
Figure 12.1 Recommended ABO type of blood components for use in ABO-incompatible bone marrow transplants. (Modified from McCullough J, Lasky LC, Warkentin PI. Role of the blood bank in bone marrow transplantation. In: Advances in Immunobiology: Blood Cell Antigens and Bone Marrow Transplantation. New York: Alan R. Liss, 1984:379–412.)

Currently used red cell storage solutions contain very little of the original donor's plasma, and so this is not usually a major source of antibody; washing red cells to remove this antibody is not necessary. If delayed red cell engraftment is occurring, the titer of anti-A should be determined and a DAT done to determine if high levels of circulating anti-A are hemolyzing newly forming donor A red cells. Although it is rare, these original patient ABO antibodies may remain for many months [105]. In making the decision when to convert to the new donor ABO type for red cell transfusion, we follow the procedure described earlier for massive transfusion by using the crossmatch to determine when all circulating antibody has disappeared. If the crossmatch technique is one that is sensitive to ABO antibodies, that is a satisfactory indicator of the safety to begin transfusing the new donor ABO type red cells.

Conversely, in a "minor" incompatible marrow transplant (e.g., O donor and A recipient), there are two dangers. First, the transfusion of large volumes of incompatible plasma with the stem cells at the time of transplant may cause hemolysis. This can be avoided by removing the plasma from the stem cell product, much the same as converting whole blood into packed red cells (see Chapter 18). The second potential problem is the beginning of production of antibody by the new donor lymphocytes while there are original patient cells continuing to circulate [106–108]. Thus, the group O donor cells may begin to produce anti-A while original patient A red cells remain, leading to hemolysis. This can be alleviated by transfusion of type O red cells. Plasma exchange transfusion is not recommended. Hemolysis of original patient type O red cells by "passenger lymphocytes" in the stem cells seems to be a greater problem in unrelated transplants, even leading to hemolysis of group O red cells as well as the incompatible A or B red cells [109].

Rh-mismatched transplants are also successful, even when Rh-positive marrow is transplanted to patients with anti-D [110]. When an Rh-positive patient receives Rh-negative marrow, some original patient Rh-positive red cells circulate for weeks after transplant. Some of these patients have developed anti-D [102, 111]. It is not known whether avoidance of Rh-positive blood components in the posttransplant period would prevent this. However, we suggest converting to the use of Rh-negative red cells at the time of transplant. When an Rh-negative patient receives Rh-positive marrow, no cases of Rh immunization have been reported. These patients can receive Rh-positive blood products after transplant. [112].

Posttransplant chimeric states

After transplantation, the development of a chimeric state (a combination of donor and original patient cells) has been reported to occur following 17% [113] and 58% [114] of cases. Thus, this is not an uncommon occurrence, and the chimeric state may be long lasting [100]. In Petz's [113] series of 172 patients, 41% of those who originally had a chimerism maintained it for 2 or more years. All other patients ultimately assumed the new donor's type.

Immune cytopenias following marrow transplantation

Because of asynchrony in the reappearance of different cell (especially lymphocyte) populations, apparent "autoimmune" cytopenias have been reported. These include autoimmune hemolytic anemia (AIHA) [111, 115], thrombocytopenia [116], and granulocytopenia [117]. These conditions have been reported after both allogeneic and autologous transplants. Only in autologous transplants would cytopenia truly be autoimmune. In allogeneic transplants, Anderson [80] proposes that the cytopenias are probably due to "transient immune system imbalance common to both allografts and autografts." In addition, either donor or recipient red cell, platelet, or HLA antibodies may reactivate soon after transplantation [118], occasionally leading to transient cytopenias of various kinds.

While ABO compatibility does not effect the clinical outcome after transplantation with PBSCs, the increasing use of reduced intensity conditioning may allow longer survival and protection of original recipient alloantibodies and could lead to clinically important posttransplant hemolysis; however, this was not observed in a small study of 25 patients [119].

Intravenous immune globulin

Since patients are severely immunodeficient after bone marrow transplantation, infections with opportunistic organisms are a major problem. Intravenous immune globulin (IVIG) is used for the prevention or treatment of some of these infections. Varicella-zoster immune globulin has been shown to prevent varicella-zoster virus infections in other immunosuppressed patients [120] and can be used if marrow transplant patients are exposed to varicella. IVIG may prevent CMV infections in bone marrow transplant patients [121], but this is not usually used because of the effectiveness of CMV-safe blood components (see Chapter 11). The largest use of IVIG in marrow transplant patients is for its potential value in reducing infections and possible role in modifying graft-versus-host disease. Because IVIG is prepared from plasma from several thousand donors, it has their antibody characteristics including antibodies against blood cells. IVIG can passively transmit red cell, platelet, and neutrophil antibodies (see Chapter 5). This can cause transiently positive red cell antibody screening and/or DATs with hemolysis, but IVIG does not seem to be a major cause of thrombocytopenia or neutropenia [122].

12.4 Solid organ transplantation

Blood component use in patients receiving solid organ transplants is modest (Table 12.9). However, during the early days of liver transplantation, transfusion episodes involving 50 to 75 units of red cells and larger numbers of other components were not uncommon [123]. Patients may require transfusion before, during, or after transplantation,

Table 12.9 Mean blood components transfused during transplant surgery (ranges of reported means in parentheses).

n^a	Organ	Red cells	Platelet concentrates[b]	FFP	Cryoprecipitate
13	Kidney	1 (0–2)	0 (0–0.1)	0	0
13	Heart	3.0 (1–6)	2.7 (0–10.5)	3.0 (0–8)	1.4 (0–5)
12	Liver (adult)	17.3 (6–37.1)	21.8 (3–58)	23.1 (11–42.5)	15.3 (0–40)
11	Liver (peds)	7.1 (1–13.7)	7.1 (1.1–23)	6.8 (0–17.2)	5.6 (0.27)
6	Lung	3.4 (1–7)	1.9 (0–6.4)	1.2 (0–2.7)	1.8 (0–5)

Source: Danielson CF. Transfusion support and complicating coagulopathies in solid organ transplantation. In: Hacke E, AuBuchon J, eds. Advances in Transplantation. Bethesda, MD: American Association of Blood Banks, 1993:45.
[a] is the number of institutions providing data. In some cases, the values supplied by the institution were estimates or a range was given. In these cases, the estimate or mean of the range has been used.
[b] Includes platelets, pheresis; each platelet, pheresis was considered equivalent to six platelet concentrates.

although the situations are not as different as in marrow transplantation and usually there are not differences in the type of blood components used at these different times.

Traditionally, kidney recipients required a considerable number of transfusions prior to transplantation because of the anemia associated with end-stage renal disease. With the availability of erythropoietin (EPO), this has decreased dramatically (see Chapter 17). During the 1960s and early 1970s, transfusions were minimized to reduce the likelihood of alloimmunization to HLA antigens and the resulting difficulty obtaining a kidney transplant. This resulted in patients being maintained at very low hemoglobin levels, which contributed to their general state of poor health. In 1973, a landmark study showed that kidney graft survival was better in patients who received transfusions than in nontransfused patients [124]. Separate studies showed that in patients receiving a kidney from a living related donor, pretransplant transfusions from the intended kidney donor were associated with improved graft survival [124, 125]. Both of these observations led to the change in practice to intentionally transfuse patients prior to transplantation [126]. This was the first clinical indication in humans that blood transfusion has an immunologic effect, and a debate is active today as to whether transfusion is associated with increased postoperative infection and increased likelihood of recurrence of malignancy (see Chapter 14).

Transfusion practice for kidney transplant patients changed again during the mid-1980s. The use of cyclosporine for immunosuppression and other improvements in patient care increased patient and graft survival rates to a level where a beneficial effect of blood transfusion was no longer apparent [127]. In addition, the growing concern about transfusion-transmitted diseases caused elimination of intentional pretransplant transfusions and in most centers a reversion to a more conservative use of transfusions. When end-stage renal disease patients awaiting transplant require

transfusion, this involves only red cells to maintain hemoglobin levels. If red cell transfusion is necessary during end-stage renal disease and before transplantation, leukocyte-poor red cells are used to reduce the likelihood of HLA alloimmunization and resulting difficulty, obtaining a compatible kidney for transplant. During the perioperative and postoperative periods, red cell transfusions may be necessary to replace operative blood loss or maintain the hemoglobin level. If complications develop involving bleeding, sepsis, etc., transfusion of other blood components can be given based on the specific needs of the patient.

Patients undergoing liver transplantation have more extensive requirements for replacement not only of red cells but also of coagulation factors and platelets. Most transplant programs use intraoperative salvage to reduce the use of allogeneic red cells. The liver disease itself usually results in coagulopathy before transplantation due to decreased synthesis of factors and slower clearance of inhibitors. In some patients, plasma exchange may be used to improve the coagulation status before surgery. The manipulations of the liver, the anhepatic phase, and the insertion of the new liver interfere with coagulation factor synthesis and cause activation of the coagulation system. There is considerable information in the literature about the changes in the coagulation system, but the impact on transfusion medicine is that there is need for coagulation factor and platelet replacement. FFP is used as the source of coagulation factors, and cryoprecipitate or fibrinogen concentrates are used as a source of fibrinogen. Some programs have planned algorithms for the use of specific combinations of blood components. Factor VIIa has not been beneficial.

Regarding heart transplants, the impact of cardiopulmonary surgery on the coagulation system and the needs for component transfusion are well known. Factors that influence hemostasis include the type of bypass instrument, the prime solution, duration of bypass, use of hypothermia, and heparinization. Usually, the coagulation alterations are not sufficient to account for excessive bleeding. Cardiopulmonary bypass also alters platelet function and number [66, 128–131]. Excessive bleeding in these patients is probably more related to platelet defects than to coagulopathy. Nevertheless, routine platelet transfusion is not necessary because most patients undergoing cardiac transplant do not experience excessive bleeding and do not require substantial numbers of red cells (Table 12.9).

CMV-negative patients undergoing solid organ transplants who receive an organ from a CMV antibody-negative donor should receive CMV-negative components (Chapter 11).

Although viable lymphocytes are administered in the transplanted organs and the patients are immunosuppressed, graft-versus-host disease is rare following solid organ transplantation. Thus, irradiation of blood components is not routine.

Blood group antibodies following solid organ transplantation

Lymphocytes in transplanted organs (passenger lymphocytes) may produce antibodies in the recipient. This phenomenon usually occurs in situations in which the donor was producing the antibodies prior to transplantation [132]. ABO is the blood group system most commonly involved. When a group O donor organ is transplanted into a group A or B recipient, the corresponding anti-A or anti-B appears in the recipient's circulation about 70% of the time; following heart–lung transplants, 40%; following liver transplants, 15%; and 15% following kidney transplants [133]. These ABO antibodies are usually IgG, appear 1 or 2 weeks following transplantation, and disappear after a few weeks. The antibodies may cause a positive DAT and varying degrees of hemolysis—in some situations quite severe [134, 135]. If hemolysis is severe, the patient can be treated with plasma exchange or preferably red cell exchange providing new red cells of the donor type.

Other blood group antibodies that have been produced by passenger lymphocytes include Rh, Kell, Kidd, and platelet antibodies [132, 136]. Usually this occurs when the donor has been previously immunized and is making the antibody at the time of transplantation.

12.5 Transfusion of patients with paroxysmal nocturnal hemoglobinura

Reports during the 1930s and 1940s of increased hemolysis following red cell transfusion in paroxysmal nocturnal hemoglobinura (PNH) patients led to the use of washed cells for these patients. It now appears that this was due to materials (e.g., anti-A or -B) in the transfused plasma that activated complement leading to hemolysis because of the absence of intrinsic complement inhibitors in PNH. This led to the belief that washed red cells must be used. Many of these early transfusions involved incompatible anti-A or anti-B, well-known complement activating antibodies. A landmark study [137] established that if transfusions are given in a way that avoids complement activation, the red cells need not be washed [138, 139].

12.6 Neonates

During the first weeks of life, the red cell mass decreases in most infants; because of the common nature of this change, it is referred to as "physiologic" anemia of infancy. Usually the minimum hemoglobin value of approximately 9 g/dL occurs at 10–12 weeks of age. However, in premature infants, there is a larger and earlier decline in red cell mass, with the hemoglobin decreasing to 7 g/dL or less. It appears that the more severe fall in hemoglobin in premature infants is caused by diminished EPO production in these patients [140]. It appears that the reduced production of EPO in response to anemia is because that in premature infants much of

the EPO is produced in the liver, which is less responsive than the kidney to hypoxia.

Because the anemia of prematurity is due to erythropoietic deficiency, it would seem that administration of EPO would be beneficial. Strauss [141, 142] summarized 14 studies of the use of EPO to treat the anemia of prematurity; he recommends that EPO be administered to infants with a birth weight between 0.8 and 1.3 kg who are in stable condition and able to take oral iron. He concluded that the data are inadequate to support the use of EPO in other premature infants and that if EPO is to be administered, it should be done as a part of a research protocol. Transfusion of very small patients presents some unique considerations that differ from those of older children [143, 144]. The anemia of prematurity is often exacerbated by blood removal for laboratory studies. The smaller the neonate, the more likely he or she will receive a transfusion. Neonates may require any of the blood components that are available for adults.

Red blood cell transfusions

Neonates may require red blood cell transfusions or exchange transfusion for hyperbilirubinemia, correction of symptomatic neonatal anemia, or acute or chronic blood loss. The decision to transfuse a newborn should be based on symptoms such as the infant's weight gain or fatigue during feeding, signs such as tachycardia or tachypnea, and laboratory values such as hemoglobin, reticulocyte count, and presence of nucleated red cells on the blood smear [145]. Although there are no exact requirements, premature infants with symptomatic anemia are usually given red cell transfusions for hematocrits of 23–25%; those with a moderate degree of cardiopulmonary disease or undergoing major surgery are transfused when the hematocrit is 30%; and those with severe cardiopulmonary disease are transfused when the hematocrit falls to 40% [141]. Each transfusion is usually 15 mL/kg. Strauss [146] found that studies comparing a liberal and restrictive transfusion strategies did not reveal a clinical benefit of one or the other. Blood drawn for laboratory tests is a common cause for red blood cell transfusion. Replacement transfusions for each blood sample collected are not recommended routinely but instead should be based on the neonate's hemoglobin value and clinical condition. Sacher et al. [147] recommend replacement transfusion when (a) more than 5% of the neonate's blood volume has been removed in less than 10 days, or (b) when there has been a 10% reduction in the infant's blood volume and the infant's initial hematocrit was 50% or greater.

Red blood cell transfusions for neonates can be given as packed red cells. Usually red cells less than 7 days of age are used to avoid transfusing supernatant containing high levels of potassium and lactic acid. However, Strauss [146] reported that several studies comparing red cells less than 7 days or up to 42 days did not show a benefit to the fresher red cells. Red cells, as currently produced by most blood banks, are suspended in a medium containing glucose, sodium, adenine, mannitol, citrate, and phosphate. Although there have been no reports of complications caused by transfusing these materials to neonates, there is very little information

on this subject. There is no specific indication for routine use of frozen, thawed, or washed red cells.

Pretransfusion testing

Because the neonate's immune system is not fully functional, the blood group antibodies in the neonate are those of the mother. Thus, a blood sample is usually obtained from both the mother and the newborn. The ABO and Rh types of both are determined and the mother's serum is tested for the presence of unexpected (non-ABO) red blood cell antibodies. If a clinically significant antibody is present, the mother's serum is usually used for subsequent compatibility testing for the neonate because her serum should have higher levels of antibody. If no unexpected red blood cell antibodies are present, no additional compatibility testing is required during that hospital stay because of the remote possibility that the neonate would form a new red blood cell antibody [148]. This approach applies if the neonate has received only group O Rh-negative red cells or red cells of group O and of the neonate's original Rh type.

Red cell products used for neonatal transfusion

Blood banks have devised several ways to provide small volumes of blood components while not wasting the remainder of the original donor unit. These techniques may involve multiple-bag systems, dispensing components in syringes, or using specially collected small units. One common approach is to collect one O-negative unit into a quadruple bag, separate the unit into four parts, and use each of these for one patient for several days. Once each of these small units is entered, it will have only a 24-hour dating period unless a sterile docking device is used. Another approach is to use one unit of O-negative red cells for all patients being transfused on that day. This makes available very fresh blood but increases the number of donor exposures for the infant. These different "minimal donor exposure" programs are described more fully in Chapter 6. Regardless of which method of storing the red cells is used, many blood banks provide the red cells to the patient care unit in syringes rather than in the plastic bag used for storage. The blood is removed from the bag through a sample site coupler. The syringe must be properly labeled and the red cells transfused soon because the syringe is not a blood storage container and the conditions and suitable storage period for these syringes have not been extensively studied. Walking donor programs are not recommended because they are associated with serious problems [149].

Techniques of administering blood to neonates

Most neonates require only a small volume of the component being transfused (usually less than 20 mL). The administration devices usually available were designed to handle large volumes of blood. Thus, the devices may lead to wastage of a large proportion of the components. Several additional factors unique to transfusion of small volumes of blood to neonates may cause hemolysis. These include flow-monitoring devices,

viscosity of the red cell units, filters, flow rates, tubing size, needle size, and pressure systems. The problem of wastage has been minimized by use of small tubing sets and infusion pumps to control the transfusion of small volumes, and most neonatal units have developed very effective methods of administering blood components. Red cells should be administered through a 23- or 25-gauge needle. It is not necessary to warm the blood routinely, but the rate of transfusion must be controlled to avoid rapid transfusion of large volumes of cold blood. Rapid transfusion of viscous red cells through small bore needles or some filters under pressure can cause hemolysis [150, 151]. Thus, careful attention to these details is important, and it is important that the blood bank has close interaction with the neonatal patient care unit to review the techniques of blood administration to ensure proper handling of the components.

CMV-negative blood components

Neonates of less than 1500 g birth weight are at increased risk of CMV infection from blood transfusions [152]. Thus, it is common to provide CMV-safe cellular blood components for such patients. However, American Association of Blood Banks Standards 10 recommend this for infants under 1200 g when the mother or infant is CMV negative and only if local data indicate that transfusion-transmitted CMV disease is a problem. For a more detailed discussion, see Chapter 11.

Irradiated blood components

Because neonates do not have a fully developed immune response, there has been concern that transfused blood components could cause graft-versus-host disease in neonates. Some physicians have recommended the routine use of irradiated blood components to prevent graft-versus-host disease in neonates. Many thousands of neonates have received transfusions of unirradiated blood components without developing graft-versus-host disease, although a few cases in neonates have been reported [153, 154]. Almost all of these patients had an underlying clinical problem that would today be an indication for irradiated blood components [155]. Because there is little evidence that this is a clinical problem, irradiation of blood components for all routine transfusions to neonates is not done in most neonatal care centers. However, irradiation of blood for neonates has been recommended for infants whose birth weight is 1250 g because of their underdeveloped immune system [156].

Transfusion of patients with T-activation

The T- or T-related antigens are present on all red cells but masked unless they are exposed by bacterially produced enzymes. Most healthy people have IgM anti-T that can cause hemolysis in some patients with exposed T-antigens. Current transfusion practice ranges from no special precautions to specific testing of at-risk patients and provision of plasma components with low or absent anti-T [157]. Clinical data are not available to support a definitive transfusion strategy [157, 158].

Platelet transfusions

Neonates may require platelet transfusions for most of the same reasons as adults and additional ones such as neonatal alloimmune thrombocytopenia, infection-related thrombocytopenia, thrombocytopenia due to maternal problems (idiopathic thrombocytopenic purpura (ITP), systemic lupus erythematosus, etc.), respiratory distress syndrome, phototherapy, polycythemia, necrotizing enterocolitis, and certain congenital diseases such as Wiskott–Aldrich syndrome. Some neonatologists prefer to administer prophylactic platelet transfusions to severely ill premature infants when the count falls below 50,000/mL [159]. Neonates can receive platelet transfusions in a volume and dose based on the size and platelet count of the patient. There are several ways to determine the recommended dose: (a) one unit of whole blood-derived platelets per 10 kg body weight, (b) four units of platelets per square meter body surface area, or (c) 10 mL of platelet concentrate per kilogram body weight. Thus, neonates will usually require less than one unit of a platelet concentrate prepared from a unit of whole blood. If a higher than usual posttransfusion count is desired, the platelet unit can be centrifuged and concentrated to provide a larger dose of platelets in the volume desired for transfusion. This "volume reduction" does not adversely affect platelet recovery or function [160, 161]. Platelets should be ABO and Rh identical with the patient.

Granulocyte transfusions

Neonatal sepsis is a rather frequent occurrence that has a mortality ranging from 3% in term infants to 90% in infants weighing less that 1 kg [162]. The abnormal function and number of neutrophils in newborns may contribute to the incidence and severity of newborn sepsis. Granulocyte transfusions have been reported to be helpful in patients who are neutropenic (less than 3000/mL) and have an inadequate marrow response [163]. Since it is often impractical to do a bone marrow examination to determine marrow reserve, some have suggested that marrow reserves are depleted if more than 70% of circulating neutrophils are immature forms [163] and that this be used as an indication for granulocyte transfusion in these patients. Strauss [162] reviewed 11 reports of granulocyte transfusions in neonates and concluded that "the role of granulocyte transfusion in neonatal sepsis is unclear." A major practical problem is that granulocyte transfusions to neonates are usually needed urgently, but lack of good preservation techniques makes them difficult to obtain because a stock inventory is not maintained. Either granulocytes obtained by leukapheresis or those separated from whole blood have been used, but the logistical problems have never been satisfactorily solved (Chapter 11), and granulocyte transfusions are not usually used for neonates.

Exchange transfusion of the neonate

Some indications for exchange transfusion in the neonate include hyperbilirubinemia, sepsis, DIC, polycythemia, respiratory distress syndrome, hyperammonemia, anemia, toxin removal, thrombocytopenia,

and sickle cell disease (SCD) [164]. In the past, hemolytic disease of the newborn (HDN) due to the Rh(D) antigen was the most common reason for exchange transfusion, and so this general discussion will focus on that situation. Most of the considerations and potential complications also apply to exchange transfusion in neonates with other conditions. The objectives of exchange transfusion in HDN are: (a) correction of anemia, (b) reduction of bilirubin concentration, and (c) replacement of Rh-positive red cells with Rh-negative cells. The use of Rh immune globulin has greatly reduced the incidence of HDN. Because there are substantial risks of exchange transfusion, considerable attention has been devoted to defining the indications for exchange. Those that apply to exchange because of blood group incompatibility are the most well described [145]. The cord hemoglobin could be one indicator, but this is not helpful because severely affected infants will have received an intrauterine transfusion affecting the cord hemoglobin value. A cord indirect bilirubin value greater than 4 mg/dL or an indirect bilirubin of 20 mg/dL within the first 72 hours of life is often used. For infants with cord indirect bilirubin values less than 4 mg/dL, the rate of change of the indirect bilirubin is another indication. Changes of 0.15–0.2 mg/dL/hour or total bilirubin of 0.6 mg/dL/hour during the first 12 hours have been used.

   The exchange transfusion is usually done via the umbilical vein. If the procedure is being carried out on a child of a few days of age, a peripheral vein can also be used. When the umbilical vessels are available, both the artery and the vein can be cannulated; then the vein can be used for transfusion and the artery for withdrawal, thus making an isovolemic exchange possible. Alternatively, the umbilical vein is used for both withdrawal and transfusion using a three-way stopcock and "push-pull" discontinuous technique. The maximum volume of blood withdrawn at one time depends on the blood volume and the condition of the infant. In a discontinuous exchange of a stable infant, aliquots of 10–20 mL may be done, but the aliquot should not exceed 5–10% of the patient's blood volume [165]. A one blood volume exchange should remove about 65% of the original intravascular constituent and a two blood volume exchange about 85%. The exchange transfusion should require about 1–11/2 hours. The red cells to be used should be ABO compatible, Rh negative, and crossmatched using the mother's serum. Mother's serum is used because the concentration of antibodies should be higher than in cord blood. Most blood banks do not have whole blood available, and thus exchange transfusion is usually done with red cells that are only a few days old. Using relatively fresh red cells is important to provide red cells containing maximum levels of DPG. Red cells as they are currently prepared are usually suspended in additive solutions containing glucose and adenine (see Chapter 5). Red cells suspended in the anticoagulant preservative CPDA-1 have been used satisfactorily for exchange transfusion [164, 166]. The initial hemoglobin, the desired final hemoglobin, and the volume intended for exchange will determine the hematocrit of the blood to be used for the exchange. The hematocrit of the blood used for exchange can be adjusted to elevate the hemoglobin in anemic patients. Formulas are

available for this calculation [166]. The red cells can be reconstituted by adding 5% normal serum albumin if it is desired to lower the hematocrit. An additional benefit of the albumin is that it binds bilirubin and thus may improve the effectiveness of the exchange transfusion, although it also increases the osmotic pressure and thus must be used cautiously. If necessary, because of coagulopathy, FFP can be used to reconstitute the red cells to provide coagulation factors during the exchange transfusion.

Another consideration due to the composition of the blood component being used for exchange is the possibility of hyperglycemia from the high glucose content of the red cell units. This may cause insulin release and a rebound hypoglycemia. Thus, blood glucose must be monitored. The citrate used as the anticoagulant in the red cell units may lead to hypocalcemia, so physicians often give supplemental calcium [164]. These infants may also metabolize citrate slowly due to poor liver function and experience acidosis, but additional buffers are not used because this may cause a later alkalosis [164].

There are a number of potential complications of exchange transfusion (Table 12.10). Because of the underdeveloped immune system in neonates, blood used for exchange transfusion in these patients should be irradiated (see Chapter 11). Also, because these patients may be poorly oxygenated, red cells used for exchange should not contain hemoglobin S. Thus, it is necessary to screen these units of red cells for hemoglobin S to avoid using blood that might have been collected from a donor with sickle trait. These potential complications can be avoided or minimized by careful technique, selection of the optimum blood component, and good general patient care.

Table 12.10 Potential complications of exchange transfusion.

Infection
Bacterial
Viral
Metabolic
Hyperglycemia
Rebound hypoglycemia
Hypocalcemia (due to citrate anticoagulant in the exchange blood)
Hyperkalemia (if older red cells are used)
Late-onset alkalosis
Hypernatremia
Cardiac
Volume overload
Cardiac arrest
Hematologic
Hemolysis
Thrombocytopenia
Neutropenia
Coagulopathy
General
Graft-versus-host disease
Hypothermia

Since many of these patients are quite ill and unstable, exchange transfusion can be a risky procedure.

12.7 Pediatric patients

Pediatric patients may require transfusion for reasons similar to those described for adults but with some additional unique situations [144], primarily in the newborn period. The other conditions leading to transfusions in pediatric patients are inherited disorders of hemoglobin, which are discussed below. Any of the blood components described for transfusion to adults may be used in children. Transfusion of red cell products may be necessary for red cell mass replacement to maintain oxygen delivery to the tissues. The discussion of the red cell transfusion trigger in adults (Chapter 11) applies also to children. However, one excellent study [167] establishes that for patients in intensive care, transfusion at a hemoglobin of 7 g/dL compared with 9.5/dL resulted in fewer transfusions with no deleterious effects. The usual dose of red cells is 2.5–5 mL per kilogram, which should elevate the hemoglobin about 1 g/dL in a stable patient. Coagulopathy may be present due to hemorrhagic disease of the newborn, DIC, massive transfusion, liver disease, major surgery, or many other conditions. If coagulation factor replacement is necessary, the usual dose of FFP is 10–15 mL/kg body weight [168]. This dose should be adequate to control bleeding in most situations unless severe DIC is present. Cryoprecipitate or fibrinogen concentrate can be used to replace fibrinogen. The usual dose of cryoprecipitate is one donor unit (bag) per 3–5 kg body weight. Another approach is one bag of cryoprecipitate per 100 mL plasma volume [168]. This should raise the fibrinogen level to 200 mg/dL, which provides adequate hemostasis for trauma or surgery.

The risks of bleeding in thrombocytopenic pediatric patients are thought to be similar to those in adults, but pediatric patients may need platelet transfusions because of maternal ITP, neonatal alloimmune thrombocytopenia, infections, maternal drug ingestion, treatment of malignancy, or congenital syndromes involving megakaryocytic hypoplasia. A whole blood-derived platelet concentrate should increase the platelet count by 8000–10,000/mL in a 70-kg adult with about 1.7 m^2 body surface area [169]. Thus, the expected response to platelet transfusion can be calculated using the formula previously described (see Chapter 11). Another approach to determining the dose of platelets is that one unit of whole blood blood-derived platelet concentrate per 10 kg recipient body weight should elevate the platelet count approximately 40,000/mL. A third alternative method of determining the dose of platelets is 1 unit per 2500 g body weight.

Techniques of transfusion

Because of the size of pediatric patients, the techniques of transfusion and the doses of components are often different from those for adults. This

means that the volumes of blood components being transfused to neonates and pediatric patients may be considerably smaller than those for adults, and the equipment for transfusion may be different. One issue is the size of the needle used for transfusion. For adults it is recommended that needles for transfusion be no smaller than 21 gauge. Hemolysis can occur from high or very low flow rates and with smaller needles, especially when older stored blood is used [150, 151, 166]. However, citrated blood stored about 24 hours has been administered through 27 gauge needles with no hemolysis [170]. Thus, the use of butterfly or angiocatheter needles of 22–27 gauge is acceptable. In patients who will receive many transfusions during a course of therapy (i.e., bone marrow transplantation or chemotherapy), indwelling catheters may be used for transfusion [165]. All blood products should be filtered, and filters with small internal volumes are now available [166]. For small patients receiving small volumes of blood, it is customary to control the rate of transfusion by using a constant-infusion or syringe pump. In general, these devices work well for this purpose [166], but the particular brand of device should be tested to ensure accuracy and lack of hemolysis. Because of the immature thermoregulatory systems in newborns and the importance of temperature control in many pediatric patients, it is often desirable to warm the blood during transfusion. A drawback of many blood-warming devices is the large volume of blood required to prime the system and the resultant loss of blood remaining in the system. If these systems are used, the volume of blood obtained from the blood bank must take into account this extra need to fill the warming device. One approach for syringe transfusion is to place the syringe in the incubator with the infant for 15–30 minutes, at which time the blood will be suitably warmed. It is important to ensure that the incubator temperature is not greater than about 37°C to avoid hemolysis. Guidelines regarding solutions for use in transfusing blood components are similar to those for transfusion of adults. An excellent summary of the policies along with specific procedures for the techniques for transfusion of neonates and pediatric patients can be found in [144, 165].

12.8 Transfusion therapy in hemoglobinopathies

Sickle cell disease
Clinical indications for transfusion

Most patients with SCD are asymptomatic even with hemoglobin concentrations as low as 6 g/dL. Thus, transfusion is not used to manage chronic steady-state anemia. Uncomplicated painful crises, minor surgery not involving general anesthesia, and minor infections are also usually not considered indications for transfusion. However, there are several situations in which red cell transfusion is indicated in these patients [171] (Table 12.11). In these situations, the goal of transfusion is to reduce intravascular sickling by diluting or replacing the patient's sickled cells with nonsickling red cells from a normal donor. The major complications of SCD are aplastic crisis, stroke, acute pain syndrome, infection (especially

Table 12.11 General indications for transfusion in sickle cell anemia.

To improve oxygen-carrying capacity and transport in patients with:
   Physiologic derangement
   Fatigue and dyspnea (usually Hb < 50 g/L)
   Acute or chronic hypoxia (PO2 < 65 mm Hg)
To decrease the concentration of HbS, thereby improving microvascular perfusion in acute
   situations such as:
   Life-threatening events including infection
   Acute, impending, or suspected cerebrovascular accidents
   Acute splenic or hepatic sequestration crises
   Acute priapism
   Acute progressive lung disease
   Fat embolization following fractures
   Preparation for surgery
   Intractable acute events, including painful crises
   Prior to injection of contrast material
To maintain HbS level below 0.3 total Hb for chronic conditions such as:
   After cerebrovascular accidents
   For leg ulcers
   During pregnancy
   For decreased performance status and disability due to recurrent acute complications in
   chronic organ failure

Source: Brettler DB. Inhibitors of factor VIII: detection and treatment. In: Hackel E, Westphal RG, Wilson SM, eds. Transfusion Management of Some Common Heritable Blood Disorders. Bethesda, MD: American Association of Blood Banks, 1992:56.

with pneumococci), acute splenic sequestration, priapism, and chronic leg ulcers. Since acute sickle cell crises rarely occur when the hemoglobin S is maintained below 45–50% of the total hemoglobin, some patients are kept on an ongoing transfusion program to maintain hemoglobin A levels between 50% and 70% of the total hemoglobin. This can usually be accomplished by transfusing 10–15 mL/kg (for children) or 1–2 units (for adults) every 2–4 weeks.

In transfusing patients with SCD, the blood viscosity must be considered as well as the proportion of hemoglobin S. When the proportion of hemoglobin is stable, the blood viscosity increases as the hematocrit increases, causing a decline in effective oxygen delivery in the hematocrit range of 20–40% [172]. Thus, in some situations, exchange transfusion may be preferred over simple transfusion.

Red cell exchange transfusion is used for acute situations in which it is desired to decrease the level of hemoglobin S and increase the level of hemoglobin A rapidly [173–175]. It is usually desirable to reduce the hemoglobin S to less than 30% of the total hemoglobin, although it is also important not to elevate the hematocrit excessively, since blood viscosity begins to increase above hemoglobin levels of 14–15 g/dL. Formulas are available for determining the volume of blood necessary to reduce hemoglobin S below 30% from various starting hematocrits [176]. Exchange transfusions can be carried out by using a three-way stopcock,

syringes, and a "push-pull" technique or by using semiautomated blood cell separators and a technique similar to plasma exchange [173–175].

Pregnancy increases the likelihood of complications and sickle cell crises. Prophylactic transfusion to maintain hemoglobin S levels below 80% of total hemoglobin is now often part of the management of these patients [177–179] although some [180–181] believe that improvements in the general care of pregnant SCD patients make prophylactic transfusions indicated only for selected patients.

Surgery—especially procedures involving general anesthesia—increases the likelihood of complications of SCD, and thus preoperative prophylactic transfusion might be effective. Preoperative transfusion can be used to reduce the hemoglobin S level to 60% or less and to elevate the total hemoglobin to about 11 g/dL [182–184]. However, the clinical benefits of these transfusions are not as clearly established as for pregnancy. If the surgical procedure is expected to be complicated or general anesthesia prolonged, transfusion is more likely to be used.

Red cell antibodies

Many SCD patients form red cell antibodies as a result of transfusion. Often multiple antibodies are present, making it very difficult to find compatible red cells [185–187]. It is difficult to determine whether SCD patients are more likely than others to form red cell antibodies because SCD patients receive many units of blood over a long period of time and appropriate control groups are not available. Immunization may also be increased because most of the red cells that the patients receive are from donors of a different ethnic or racial group with some differences in antigen frequency, thus leading to the patients' being exposed to antigens that they lack [185]. Garratty [186] concludes that red cell antibody formation is more common among SCD patients, but regardless of this point, there is some debate about the selection of donors for SCD patients. Since most SCD patients do not form red cell antibodies, standard blood bank operations—in which no special donor-recipient matching is done—can be followed, and when red cell antibodies occur, appropriate antigen-negative red cells are used [187, 188]. However, locating antigen-negative red cells may be quite difficult for some patients with multiple antibodies, and this can delay transfusion. An alternative strategy is to avoid antibody formation by using red cells matched for the antigens that are most likely to cause immunization [185, 189–191]. Using a "limited" phenotype matching program involving ABO, D, C, c, E, e, and Kell would prevent 53% of antibodies and an "extensive" phenotype matching program (limited plus S, Fya, and Jka) would prevent about 70% of antibodies [192]. Although the optimum strategy has been debated for two or three decades, there is no general agreement, but most large SCD centers use some form of limited match. For nonimmunized patients, matching for C, E, and K is recommended and should be practical because this should represent about 13% of the donor population [171]. For immunized patients, additional matching for S, Fya, and Jkb has been

proposed, but only 0.6% of donors have this phenotype [171] so this is very problematic.

Transfusion reactions

Transfusion reactions may be confusing in SCD patients because some symptoms of a transfusion reaction such as fever, sickle cell crisis pain, or arthralgias may be caused by the SCD. Since some patients have multiple antibodies, making it difficult to find compatible red cells, there may be additional concern as to whether acute or delayed hemolysis is occurring. A particular issue in SCD patients is the occasional occurrence of hemolysis in the absence of demonstrable red cell antibodies [186, 193–196] or the occurrence of more severe anemia than was present before transfusion. This latter phenomenon is referred to as "hyperhemolysis" or "bystander immune hemolysis" [193, 194, 196]. Thus, hemolysis of red cells that do not contain the antigen involved in the transfusion reaction could be due to activated complement components, autoantibody formation, or increased susceptibility of red cells to complement-mediated lysis. SCD patients may have delayed transfusion reactions or rapid clearance of transfused red cells in the absence of detectable red cell alloantibody. This may be due to the intravascular environment in SCD patients resulting in accelerated clearance of apoptotic red cells [197]. There is considerable benefit to avoiding febrile nonhemolytic transfusion or other types of reactions that can be confused with or mask the symptoms of SCD. Thus, leukodepleted blood components are recommended.

Components

Standard red cell components are satisfactory for transfusion to SCD patients. For acute crises, it may be desirable to use red cells less than 1 week old to provide maximal oxygen-carrying capacity immediately after transfusion. It is preferable to avoid transfusing sickle-trait red cells, especially during a crisis. The hypoxia and acidosis in the patient may be adequate to cause sickling of the sickle-trait red cells, thus adding to rather than alleviating the problem. In addition, the combination of homozygous and heterozygous sickle hemoglobin and hemoglobin A may complicate monitoring the results of transfusion. Thus, red cells to be used for SCD patients should be free of sickle-trait hemoglobin.

Thalassemia

Children whose hemoglobin is maintained at 8 g/dL or greater have better growth, less hepatosplenomegaly, fewer bone abnormalities, and less cardiac enlargement. This has led to the use of "hypertransfusion," in which the hemoglobin is maintained at 9 g/dL or greater, [198, 199] or "supertransfusion" programs, which maintain the hemoglobin at 11.5 g/dL or greater. The supertransfusion program is thought to almost completely suppress the patient's own hematopoiesis without increasing the transfusion requirements after the initial transfusion [200] although others believe supertransfusion will increase iron overload [143] and may not provide an overall benefit. Transfusion requirements for thalassemia patients usually are 80–150 mL/kg per year [201].

Ordinary red cells can be used for the transfusions. There has been some question of whether the reduced 2,3-DPG in stored red cells would result in tissue hypoxia and increased production of affected red cells, thus partially negating the value of the transfusion. There is little evidence that this actually occurs. A more common problem is the development of febrile or allergic transfusion reactions in these multitransfused patients. The febrile reactions can be avoided by using leukocyte-depleted red cells. Allergic reactions are usually caused by plasma proteins and, if not controlled by antihistamines, may necessitate the use of washed red cells.

Red cell alloantibodies

These multitransfused patients may, as expected, develop red cell alloantibodies [187, 202]. The incidence ranges from 3% to 35% with 13% having three or more antibodies [200]. Autoantibodies can also develop causing more severe hemolysis than suggested by the alloantibodies present [202]—a situation similar to that seen in SCD. The considerations about whether to provide red cells matched for some antigens or to await the development of antibodies is similar to those for SCD patients. Also, HLA alloimmunization can develop, leading to nonhemolytic febrile transfusion reactions, and so leukocyte-depleted red cells are recommended.

12.9 Hemophilia and von Willebrand's Disease

Hemophilia A (Factor VIII deficiency)

The blood components and derivatives containing coagulation factors VIII and IX are described in Chapter 2, and the use of these products to replace coagulation factors is also discussed in Chapter 11. For patients with mild hemophilia, minor procedures or dental work may be managed using desmopressin acetate to stimulate endogenous release of factor VIII [203]. In more severe cases or for major bleeding episodes or major procedures, factor VIII concentrates are used. For early treatment of minor bleeding episodes, patients can keep factor VIII concentrates at home and are taught self-injection techniques. This early treatment of bleeding episodes minimizes the complications and greatly increases convenience for the patient.

About 15% of patients with severe hemophilia develop inhibitors that are alloantibodies that inhibit the procoagulant activity of the factor VIII molecule. These antibodies react with different epitopes on the factor VIII molecule [203]. The treatment of bleeding episodes in hemophilia patients with inhibitors depends on the severity of the bleeding episode, the severity of the hemophilia, and the response of the patient to factor VIII [204]. The different alternatives for treatment are shown in Table 12.12.

Hemophilia B (Factor IX deficiency)

For the treatment of factor IX deficiency or hemophilia B, coagulation factor concentrates containing factor IX are available, and this has

Table 12.12 Alternative strategies for management of Factor VIII inhibitors.

Large doses of factor VIII
Activated or nonactivated prothrombin complex concentrates
Plasma exchange
Porcine factor VIII
Recombinant factor VIIa
And/or immunosuppression with cytotoxic agents or IVIG, 177
Antibody depletion using a staphylococcal protein A column

simplified treatment of these patients. Inhibitors occur more frequently in factor VIII-deficient than in factor IX-deficient patients.

von Willebrand's disease

von Willebrand's factor is present in the plasma in a wide range of multimers. The high-molecular-weight multimers are the most hemostatic. Patients with von Willebrand's disease can be managed either by stimulation of endogenous production of von Willebrand's factor in those mildly affected or by replacement of the deficient coagulation factor in those severely affected. The former is accomplished by the administration of desmopressin acetate. The blood component used to replace von Willebrand's factor is cryoprecipitate. Each bag of cryoprecipitate contains a substantial amount of von Willebrand's factor, and so the usual dose is 1 unit per 10 kg body weight every 12 hours. Cryoprecipitate is preferred over FFP because of the higher content of high-molecular-weight multimers in cryoprecipitate. A vWF concentrate is now available (Chapter 5).

12.10 Autoimmune hemolytic anemia

There are several types of AIHA (Table 12.13). The direct antiglobulin (Coombs) test is one of the hallmarks in the diagnosis of AIHA. In warm antibody AIHA, the DAT is usually IgG but may be IgG + C3 or infrequently C3 only. In mixed AIHA, the DAT is almost always IgG + C3 and in cold agglutination disease it is positive with anti-C3. When a patient is found to have a positive DAT, an eluate should be prepared and tested against a panel of reagent red cells to determine whether any antigen specificity is present.

Decision to transfuse

When these patients require transfusion, it is time consuming to find compatible red cells, and after considerable effort, all red cell units may be incompatible. Therefore, the laboratory work to locate red cell units suitable for transfusion should be initiated early in the patient's course, even if at the time transfusion does not seem to be indicated. If possible, a larger than usual blood sample should be obtained and saved for possible future use. The decision to transfuse a patient with AIHA should be based

Table 12.13 Autoimmune hemolytic anemia.

IgG warm antibody type
Infections
    Postviral autoimmune hemolytic anemia of childhood
    Infectious mononucleosis
    HIV-1 infection
Immune deficiency states
    Hypogammaglobulinemia
    Wiskott–Aldrich syndrome
    Dysgammaglobulinemia
Neoplasms of the immune system
    Lymphocytic disorders
    Chronic lymphocytic leukemia
    Non-Hodgkin's lymphoma
    Hodgkin's disease
    Plasma cell disorders
    Waldenström's macroglobulinemia
    Multiple myeloma
Diseases of immune dysfunction
    Systemic lupus erythematosus
    Scleroderma
    Rheumatoid arthritis
    Sjogren's syndrome
    Immune thrombocytopenia (Evans' syndrome)
Miscellaneous diseases
    Pernicious anemia
    Thyroiditis
    Ulcerative colitis
    Ovarian tumors and cysts
    Other tumors
Pregnacy
Drug-related
Idiopathic

IgM warm antibody type
Idiopathic
Lymphocytic malignancies

IgM cold antibody type
Infections
    EBV-infectious mononucleosis
    Mycoplasma pneumoniae
    Listeria monocytogenes
Idiopathic
Malignancies
    Lymphocytic malignancies
Adenocarcinomas
Benign monoclonal B-cell gammopathy

Source: Adapted with permission from Rosse W. In: Handin RI, Lux SE,
Stossel TP, eds. Blood: Principles and practice of hematology.
Philadelphia: J.B. Lippincott Company, 1995:1831.

on the severity of the anemia, whether the anemia is rapidly progressive, and the associated clinical findings. The hemoglobin may change much more quickly in AIHA compared with chronic anemia patients. Therefore, in newly diagnosed patients with AIHA, the hemoglobin should be measured as frequently as every 4 hours to determine whether the anemia is progressing. Transfusion is not recommended unless the hemoglobin is in the range of 5–8 g/dL. Many of these patients will compensate for their anemia, especially on bed rest in the hospital, and transfusion is not necessary. When the anemia becomes severe, cardiovascular or neurologic symptoms are those usually seen. This may involve angina, cardiac failure, mental confusion, weakness, or obtundation. Transfusion is indicated for such patients. Although the concept has been developed to avoid transfusion in AIHA patients, the indication for transfusion should be only slightly more conservative than for other patients, and patients should not be put at risk just to avoid transfusion [205].

Red cell typing

In the serologic investigation of AIHA patients, ABO and Rh typing can usually be done without difficulty, although proper controls must be used, especially with the Rh typing, to ensure that the results are not obscured by spontaneous agglutination caused by the autoantibody. There are particular problems in the serologic investigation of patients with cold antibody-type AIHA. The cold-reacting autoantibody may cause red cell agglutination even at warmer temperatures, thus making the routine test methods inaccurate. If the patient has not been transfused recently, it is advisable to type his or her red cells for other clinically significant antigens as an aid in identifying any alloantibodies potentially present [206–210]. Knowledge of the antigens that the patient lacks will also aid in the identification of antibodies that may form in the future. The development of nucleic acid-based blood typing systems may be of great help.

Serologic investigation of AIHA

The serologic evaluation of sera containing autoantibodies is to determine whether any alloantibodies are present and whether the autoantibody has any specificity. Several serologic techniques are also available to detect alloantibodies that might be present from previous transfusions or pregnancy. These techniques use two approaches: removing the autoantibody from the patient's red cells to determine which antigens the patient lacks and thus might be immunized against; and absorbing the patient's serum using autologous red cells or those known to possess certain combinations of antigens to try to identify an underlying alloantibody. These techniques are discussed in Chapter 10. The possibility of drug-related AIHA should be considered in the serologic investigation [209]. If any clinically significant alloantibodies are present, red cells selected for transfusion should lack the corresponding antigen.

Removing autoantibody from the patient's red cells

This can be done by treating the DAT-positive red cells with chloroquine or a combination of acid glycine and EDTA (see also Chapter 9). This makes

it possible to phenotype the patient's red cells to determine the potential for alloantibody formation. Kell system antigens cannot be detected if the acid glycine/EDTA method is used, but all other red cells antigens can be detected after either red cell treatment.

Warm reactive autoantibodies

It is important to test the serum for the presence of alloantibodies because they have been found in up to 40% of patients with AIHA [207, 210]. One method used in the past but no longer recommended is dilution of patient's serum and retesting it against a panel. It was believed that the autoantibody would be diluted leaving any stronger and, thus, identifiable alloantibodies [211]. However, there is no reason to believe it, and data do not support this concept. Absorbing the sera is the preferred approach.

Heat or other elution techniques can be used to remove autoantibody so that autologous cells can be used to absorb the autoantibody from the patient's serum, leaving behind any alloantibody present. It is preferable to use a reagent called ZZAP to treat red cells to absorb the autoantibody, leaving behind any alloantibody present. This autoabsorption technique can be confused if the patient is recently transfused [212]. Alternatively, allogeneic panel cells can be used for the absorption. The cells may be used untreated or treated with enzymes, ZZAP, or polyethylene glycol, which enhances antibody absorption. Two or three cells are selected such that their phenotypes enable an experienced red cell serologist to determine whether alloantibodies are present in the absorbed serum.

Crossmatching

If transfusion is indicated, the two goals of compatibility testing are to select red cells that will survive at least as long as the patient's own cells and to avoid transfusing red cells that are incompatible with any clinically significant alloantibodies the patient may have. If the patient has circulating antibody (as well as bound), the crossmatch with native serum will be incompatible. Some blood banks crossmatch the patient's serum against several donor units and select the least incompatible one(s). As long as the difference in reactivity is not due to an alloantibody, there is no evidence that this difference in reactivity indicates a different clinical effect [208]. It is preferable to do the crossmatch with absorbed serum to minimize the chance of alloantibody–antigen reactivity.

The frequency of repeating the antibody identification studies is not standardized. For patients with known alloantibodies, methods must be used to identify additional clinically significant antibodies, and this should be done on each new blood sample if the patient has been transfused since the previous sample. Because up to 40% of patients may form alloantibodies [207], continued testing of sera even in patients without known alloantibodies is warranted. The decision to retest the patient's serum can be similar to that used for patients who have alloantibodies but not AIHA (see Chapter 10).

When the serologic investigation has been done in a reference laboratory, donor units will have been selected and crossmatched there and

the hospital may or may not wish to repeat the crossmatch. There seems little point to this if the hospital does not use the same serum sample and technique as the reference laboratory or create their own similar sample by repeating the manipulations done in the reference laboratory. Most hospital blood banks have established relationships and trust with their reference laboratory and, thus, do not repeat this testing and crossmatch.

Selecting specific red cell units

Selecting the red cell units that will have optimum survival may be difficult. The patient's serum will react with red cells from all donors because the autoantibodies have a broad spectrum of reactivity. The autoantibody may obscure alloantibodies present in the patient's serum. It is usually not possible to obtain red cells for transfusion that are compatible (negative crossmatch). The risk is that the broad, nonspecific reactivity will obscure an alloantibody, so laboratory testing is designed to detect this problem and select units that lack the antigen corresponding to any alloantibody present. In the absence of an alloantibody, the nonspecific reactivity from the patient's autoantibody makes the crossmatch incompatible but usually does not cause red cell destruction in excess of that already occurring because of the patient's disease. The patient's serum is also studied to determine whether the autoantibody has any blood group specificity. If the autoantibody has blood group specificity, selection of donor red cells lacking the corresponding antigen may provide cells that survive better than the patient's, although the data to support this practice are not convincing. Even when the autoantibody has blood group specificity, it is not always possible to provide red cells that lack the corresponding antigen. For instance, if the autoantibody has anti-e specificity and the patient is Rh-negative, it will be difficult to locate Rh-negative red cells that lack the e antigen (e.g., phenotype cdE/cdE) because this is an unusual Rh phenotype. Instead the common Rh-negative phenotype cde/cde would be used even though the autoantibody has e specificity.

Because of the autoantibodies, red cells from all donors will have a shortened survival. Despite this hemolysis, patients with AIHA do not require unusual or special red cell components. The usual packed red cells are satisfactory. It is advisable to choose units that are not toward the end of their storage life so as to obtain the maximum benefit from the transfusion by avoiding loss of senescent red cells. It is also advisable to use leukocyte-depleted red cells to avoid a possible febrile transfusion reaction, which might be confused with a hemolytic reaction and might (at least temporarily) interrupt the transfusion. There is no reason to use frozen deglycerolized red cells for AIHA patients.

Although AIHA patients are experiencing hemolysis, they usually do not experience signs or symptoms of an acute hemolytic transfusion reaction. However, transfusion in patients with AIHA involves risks in addition to those usually associated with transfusion. The unique potential complications of transfusion in AIHA patients are increased hemolysis and congestive heart failure. The increased hemolysis can occur not because the rate increases but because red cell destruction is a mass-action

phenomenon, and the number of red cells (red cell mass) destroyed increases as the total-body red cell mass is increased by transfusion. Transfusion may also cause congestive heart failure due to circulatory overload if the patient has poor cardiac reserve.

The serologic evaluation is different in patients with cold-type AIHA compared with those with warm-type anemia; however, the considerations in transfusing these patients are similar. One unique issue for patients with cold-type anemia is the need to be sure that the blood is warmed before transfusion to avoid hemolysis due to the cold active antibody. Blood warming is discussed in Chapter 13.

12.11 Pregnant women

Anemia is common during pregnancy; however, these patients rarely require transfusion. Very few (0.87% in one study) women require transfusion for post partum hemorrhage. Of this small percentage, only one-third required blood components other than red cells and in more than 12,000 deliveries there were no deaths or organ dysfunction as a result of hemorrhage [213].

When transfusion is necessary, it is usually because of some other complicating factor or disease. The choice of blood components then is based on the specific reason for the transfusion (i.e., acute blood loss, SCD, etc.). It is recommended that pregnant patients who are CMV negative or whose CMV status is unknown receive CMV-free blood components because of the severe effects that acute CMV infection of the mother can have on the fetus (see Chapter 11).

12.12 Acquired immune deficiency syndrome

The hematologic complications of acquired immune deficiency syndrome (AIDS) are anemia, thrombocytopenia, leukopenia, lupus-like coagulation inhibitors, and DIC caused by either the disease itself or the antiviral agents used for therapy.

Anemia
The anemia in AIDS patients is similar to the anemia of chronic disease; it is characterized by a low reticulocyte count and adequate iron stores. Anemia is probably due to ineffective erythropoiesis, possibly because of the effect of HIV infection on erythroid maturation or the release of cytokines that inhibit erythroid maturation [214,215]. The decision to transfuse these patients is similar to the decision for other patients with chronic anemia and is based on symptoms and hemoglobin level. When red cell transfusion is used, standard red blood cells are satisfactory. The use of irradiated blood components for AIDS patients is not necessary (see Chapter 11). Transfused cells do not lead to sustained microchimerism [216]. Thus, HIV infected patients do not seem to be at risk for transfusion-associated graft-versus-host disease [216].

Thrombocytopenia

The thrombocytopenia in AIDS patients may be due to increased platelet-associated immunoglobulin [217] circulating immune complexes bound to the platelet surface [217], antibodies that cross-react with platelet surface glycoproteins [218], or an effect of the viral infection of megakaryocytes [219]. Because in general the thrombocytopenia is thought to be on an immunologic basis, platelet transfusions are generally not indicated. Instead corticosteroids, immunoglobulins, or splenectomy are preferred treatments in patients whose thrombocytopenia poses a major problem. Platelet transfusions may be used in bleeding emergencies, as would be the case for autoimmune ITP.

Leukopenia

The lymphocyte, neutrophil, and monocyte-macrophage cell lines may all be affected in AIDS patients. However, transfusion therapy for these leukopenic situations is not generally used.

Lupus anticoagulant

AIDS patients with lupus anticoagulant should be managed as any other patient with a lupus anticoagulant. Prophylactic treatment is not necessary.

Disseminated intravascular coagulopathy

AIDS patients with DIC should also be managed like others with DIC. If the process is severe, plasma exchange is indicated. These patients may pose a potential problem during plasma exchange because of the known infectious plasma. This is discussed more fully in Chapter 19.

12.13 Transfusing patients with IgA deficiency

Because IgA deficiency is the most common human immunodeficiency, this must be present in many transfusion recipients. About one-third of IgA-deficient patients develop anti-IgA [220]. Also, many individuals do not have a complete deficiency of IgA but have low levels. Thus, the IgA antibodies may be of limited or broad specificity. It is thought that the broad specificity antibodies found in the absence of IgA are those responsible for severe anaphylactic reactions that occur when these patients are transfused with IgA-containing blood products (Chapter 14) [221–223]. However, there is not universal agreement on the clinical relevance of IgA antibodies [222–224] and it appears that the presence of IgA antibodies is a poor predictor of an adverse reaction. Thus, transfusion services do not screen patients prior to transfusion to identify IgA-deficient patients. If a patient has an anaphylactic-like reaction, their IgA levels are measured and, if low or absent, a test for anti-IgA is done. Patients with anti-IgA are usually given blood products lacking IgA. This is easy for red cells because they can be washed to remove remaining plasma, but plasma products and platelets must be obtained from IgA-deficient donors, which most large blood centers have available. Different IVIG brands have

different levels of IgA and one with low levels is used. Most IVIG products contain small amounts of IgA. Salama et al. were able to prevent anaphylacic reactions in IgA deficient patients by preincubating the IVIG with autologous plasma from the patient that contained anti-IgA. This eliminated the anaphylacic reactions in this patient [225].

There are usually two difficulties with these patients. First, because of the unclear role of anti-IgA, a lively debate usually occurs as to whether to use IgA-deficient blood products. This is easy if the patient is expected to have a one-time use of a few red cells such as elective surgery. Even then, this labels the patient in blood bank records and sets a precedent for future transfusions. The second difficulty is if the patient is undergoing a therapy such as hematopoietic cell transplantation that will require a large number and variety of blood components. Use of only IgA-deficient products becomes a major effort for a situation in which its value is unclear. What usually happens is that the IgA-deficient blood components are used only for patients who have had an anaphylactic type reaction, or are IgA-deficient, and have anti-IgA.

Because IgA deficiency is rather common and up to 40% of IgA deficient individuals are reported as having anti-IgA, there is a possibility that transfusion of anti-IgA might cause reactions. However, transfusion of apheresis platelets from IgA deficient donors with anti-IgA does not appear to be associated with a great frequency of reactions [226].

12.14 Autoimmune thrombocytopenia

Autoimmune thrombocytopenia patients have platelet IgG autoantibodies that bind to platelets and cause accelerated platelet destruction by interacting with Fc receptors on macrophages [227]. This also results in a severely shortened intravascular survival of transfused platelets. Despite very low platelet counts, most patients with autoimmune thrombocytopenia do not require platelet transfusion. It has been suggested that these patients have a population of young platelets, which provide better hemostasis than would be expected based on their platelet count [228]. For instance, when autoimmune thrombocytopenic patients undergo splenectomy, platelet transfusions are rarely required [229]. There are many therapies available for these patients, including corticosteroids, splenectomy, the androgen danazol, vinca alkaloids, immunosuppressives such as cyclophosphamide or azathioprine, IVIG, and recently factor VIIa. The intravenous form of Rh immune globulin (Rh-IVIG) may also be effective in Rh-positive patients [230]. The mechanism of action of RhIG, its effect in Rh-negative patients, or splenectomized patients is not known [231], but hemolysis leading to the need for red cell transfusion can occur [232, 233]. In general, patients are treated with corticosteroids and if they fail to respond, IVIG or intravenous RhIG are used [227]. The major risk is for intracranial or intra-abdominal bleeding, which rarely occurs when the platelet count is above 20,000/μL [227]. Although the survival of transfused platelets is reduced in these patients, platelet transfusion should

be administered if serious life-threatening hemorrhage occurs, even though the transfused platelets may survive for only a few minutes or hours [227, 234]; two to three times the usual dose of platelets should be used [227]. Some of these transfusions will cause a short but measurable increase in the platelet count [235]. Plasma exchange is not helpful, and splenectomy is usually done only as a last resort.

12.15 Neonatal alloimmune thrombocytopenia

Neonatal alloimmune thrombocytopenia is estimated to occur in 1 in 5000 live births. It is the platelet analog of HDN [236, 237]. In neonatal alloimmune thrombocytopenia, the mother becomes immunized to an antigen that she lacks but the fetus has inherited from the father. Alloimmunization is usually due to the platelet antigen HPA1 (PlA1), but many other platelet-specific antigens have been involved [238] (see Chapter 9). Maternal IgG antiplatelet antibodies then cross the placenta and cause thrombocytopenia in the fetus [239]. Mortality rates of approximately 15% have been reported [240] primarily due to intracranial hemorrhage either before birth or during vaginal delivery. Platelet antibody testing is recommended for mothers who have previously delivered an affected infant. Testing of sera of pregnant women at risk of delivering a baby with neonatal alloimmune thrombocytopenia is helpful if an antibody is detected, but failure to find platelet antibody does not ensure that the baby's platelet count will be normal [241, 242].

   If neonatal alloimmune thrombocytopenia is diagnosed during pregnancy, fetal platelet counts can be obtained. If the fetus is severely thrombocytopenic, the mother can be treated with IVIG [241]. If necessary, intrauterine platelet transfusions can be given [243], although this is not usually necessary because the fetus is usually not at risk until labor begins. If thrombocytopenia is present at the time of delivery, a cesarean section can be done to minimize the trauma to the infant. If the infant is severely thrombocytopenic at birth with a platelet count below 50,000/mL or serious hemorrhage, treatment should be initiated. Treatment can involve transfusion of platelets lacking the offending antigen or exchange transfusion to remove the offending antibody. Platelets lacking the antigen can be obtained from the mother, although the plasma containing the antibody should be removed before transfusion and the platelets resuspended in saline or AB plasma [241]. If platelet transfusion is urgently needed and antigen negative platelets are not available, antigen positive platelets may be temporarily helpful [244]. Because of the small size of the infant, an adequate number of platelets can be obtained from one unit of whole blood; apheresis is not necessary. However, it may be difficult for the mother to donate a unit of blood because of postpartum anemia or logistic reasons if the infant has been transferred to another hospital. But on the other hand, a more liberal transfusion policy may be valuable for patients who cannot be weaned from mechanical ventilation after six days. Most large blood banks have a

few HPA-1a-negative donors available to provide compatible platelets if laboratory facilities are available to quickly determine the serologic specificity of the antibody or if this is known in advance. The half-life of IgG is approximately 21 days, and so more than one platelet transfusion may be necessary in severely affected infants until maternal antibodies remaining in the infant decline. Alternatively, exchange transfusion can be done using techniques similar to those recommended for HDN. However, since it is impossible to remove all of the antibody, and there can be serious complications from exchange transfusion, management using platelet transfusions is usually preferable to exchange transfusion.

12.16 Neonatal alloimmune neutropenia

This is the neutrophil analog of HDN and neonatal alloimmune thrombocytopenia. It is extremely rare; only a few cases have been reported in the literature and there are no estimates of its incidence. Patients usually are discovered because they develop infection of a circumcision site or the perineal area. Cases due to several different neutrophil-specific antibodies have been reported [245]. Although these infants can be given granulocytes obtained from a whole blood donation by the mother, the very short half-life of granulocytes limits the effectiveness of this approach. Thus, exchange transfusion is the recommended approach for these patients. Laboratory testing for neonatal alloimmune neutropenia is discussed in Chapter 9. Neonatal neutropenia can also be caused by placental transfer of maternal neutrophil autoantibody [245, 246].

12.17 Autoimmune neutropenia

Autoimmune neutropenia (AIN) in children is usually primary and resolves spontaneously [247, 248] and in adults is usually secondary. Laboratory testing for AIN is described in Chapter 10. Granulocyte transfusion is not considered a helpful therapy in these patients.

12.18 Rare blood types

There is no universal definition of a rare blood type. For clinical transfusion purposes this usually refers to an individual who lacks a blood group antigen that is present in very high frequency in the normal population. This means that the individual will almost certainly be exposed to the antigen if he or she receives a transfusion or becomes pregnant. Almost all of these patients will have circulating antibody against the antigen, which is what brings them to the attention of the blood bank. Thus, the first question is whether the antibody is clinically significant and likely to cause accelerated destruction of red cells. In general, antibodies that react in vitro at body temperature (37°C) may be clinically significant,

although many such antibodies are not. Reference texts should be consulted in making the decision. Use of an in vivo crossmatch or monocyte monolayer assay may be helpful (see Chapter 10). If the antibody is clinically significant, efforts should be made to obtain red cells that lack the antigen. If such compatible donors are not available through the local blood supplier, donors can be sought from the American Association of Blood Banks and the American Red Cross, who maintain national registries of rare blood donors. Red cell typing of relatives of the patient can be done as another strategy to locate antigen negative red cells. Considerable planning may be necessary to obtain the red cells, especially if the donors live in other cities. If the transfusion is to replace blood loss during elective surgery, it may be necessary to plan the surgery around the availability of blood. Red cells from some rare donors may be available only in the frozen state, which may create additional problems if the transfusion is for anticipated but uncertain blood loss. To avoid wasting rare red cells and also avoid transfusing the patient unnecessarily, close communication between the blood bank and the clinician is essential.

   If the need for transfusion is urgent, it is helpful to consider in greater detail the likely clinical effect of the antibody. If the antibody can be expected to cause a somewhat shortened red cell survival but little or no acute hemolysis or symptoms, the decision might be made to use incompatible red cells, at least to maintain the patient until antigen-negative compatible red cells can be located.

   Patients with rare blood types who do not have an antibody occasionally become known to the blood bank. For those patients, red cells negative for the rare antigen of concern are not necessarily provided. The decision to use antigen-negative red cells for nonimmunized patients should be based on the clinical significance of the antibody the patient might produce, the antigenicity of the antigen, the patient's current medical condition, and the likelihood that the patient will require transfusion or may become pregnant in the future.

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13 Techniques of Blood Transfusion

Great care is taken to collect blood from suitable donors, produce components, use appropriate effective tests, and store them under controlled conditions. The final stage of transfusing the blood component must also be done carefully so that the transfusion will be as safe and effective as possible.

Transfusion-related fatalities other than those due to disease transmission are estimated to occur once in 100,000 patients who receive a transfusion or once in 800,000–1,100,000 transfusions [1]. The most common cause of transfusion-related fatality reported to regulatory agencies is hemolytic transfusion reaction [1,2] due to ABO incompatibility, and this is usually the result of "management" error [1,2]. One in 12,000 units of blood is administered to the wrong patient [2]. The incidence of ABO-incompatible transfusions is one in 10,000–40,000 [1–4]. Most of these management errors occur outside of the blood bank in the process of obtaining blood samples or administering the transfusion (Table 13.1) [1–4]. "Blood given to the wrong person" is the most common of all the errors [1]. This is not a problem resulting from the urgent need to transfuse patients during surgery, since about two-thirds of these errors occurred outside of the operating suite.

In order to minimize errors, it is essential to have clearly defined procedures that are well understood and carried out by qualified personnel. Each hospital should have its own specific blood administration procedures and should designate the individuals authorized to administer blood components. Blood components are prescription drugs and thus can be administered only on the written order of a physician. The physician's order should include the blood component to be administered, any special requirements, the rate of administration, and any other instructions for care of the patient during the transfusion.

13.1 Obtaining consent for transfusion

Once it has been determined that a blood transfusion is necessary, the procedure should be explained to the patient to minimize his or her apprehension and to obtain the patient's consent. In the past, a discrete consent for transfusion was usually not obtained. Acquiring consent for documentation of informed consent for transfusion in the medical record

Table 13.1 Types of errors leading to transfusion-related fatalities.

Failure to properly identify the recipient when the blood sample is collected for
     compatibility testing
Incorrect labeling of the properly collected sample
Improper labeling of tubes when testing is carried out in the laboratory
Use of an incorrect sample in the laboratory
Recording results in the incorrect record within the laboratory
Placing the compatibility tag on the incorrect unit
Releasing the unit to the incorrect patient
Administering correctly labeled and tested unit to the incorrect patient

Source: From information in Sazama K. 355 reports of transfusion-associated deaths.
Transfusion 1990; 30:583; and Linden JV, Paul B, Dressler KP. A report of 104 transfusion
errors in New York State. Transfusion 1992; 32:601–606.

is under consideration by the Joint Commission [5]. The elements of the
consent are rather standard and include the nature, severity, and
probability of risks and the general time frame in which they can be
expected [6, 7]. An additional step that can occur along with the consent
process is the documentation of the reason for transfusion, which is also
now under consideration by the Joint Commission [5]. This is also
important, as lawsuits may contend that a transfusion that caused a
complication may not have been necessary. Obtaining consent also
provides an opportunity to describe the alternatives to transfusion as now
required in some states or the ramifications to refusing a transfusion [6, 7].
There is great variation in how consent is obtained. This ranges from the
physician discussing transfusion with the patient, to use of printed
material, to nurses discussing transfusion with the patient, to
incorporation of information in general consent materials, or inclusion of
information in care protocols. The process may be documented by a note
in the medical record, a discrete consent form, or as part of another
consent form such as that used for consent for a surgical procedure.
Although a single approach cannot be defined, it is clear that physicians are
expected to ensure that patients understand the risks of transfusion and
any alternatives to homologous donor blood that might be available.

13.2 Obtaining the blood sample
for compatibility testing

One of the most common errors that leads to the administration of
incompatible red cells is mislabeling the blood sample to be used for
compatibility testing [1, 2, 4, 8]. Labeling errors range from 1 in 2900 to 1
in 6000 samples, and patient identification and specimen labeling errors
occur 1:15,000 to 1:30,000 of which 70% are bedside errors [4]. A more
recent study reports the disturbing observation that during a 30-day
period the aggregate mislabeled sample rate in 122 hospitals was 1.12%
[9, 10]. Mislabeled blood samples are responsible for 10–20% of blood

being administered to the wrong patient [1, 9]. Three basic identification systems used for specimen collection and control are wristbands, dedicated transfusion systems, and mechanical barriers. In the wristband system, the hospital wristband and unique hospital number are the basis for identifying of the patient and relating laboratory work and the transfusion of blood components. Unfortunately, errors in the availability or accuracy of wristbands are common, occurring on average in 5% of wristbands and varying from 1% to 60% in different hospitals [10]. These errors include absence of the wristband or errors in the information it contains. In the dedicated transfusion system, a separate wristband, request form, and labels are associated with unique preprinted numbers linking all of these items. In the mechanical barrier system, a unique number is assigned to the patient, and this number must be entered into a device that opens a mechanical barrier to the use of the unit. Although this system has been reported to reduce potential transfusion accidents [10], it has not become widely used probably because it still requires that staff members write the unique number on tubes being obtained for compatibility testing. Several computer-based systems that generate tube labels are being implemented, but there is little data to determine their impact on labeling errors. To reduce misidentification of the recipient, a network-assisted transfusion management system has been shown to be effective [11] and use of a blood administration assessment program (FOCUS-PDCA] improved the processes of requesting and administering blood products [12]. However, neither of those is in wide use.

Each hospital should have a specific written procedure for obtaining blood samples, and individuals collecting blood samples for compatibility testing should be familiar with and follow that procedure. Typically, this involves the person collecting the sample checking the identifying information on the patient's wristband against the blood request form. Charts or tags on the bed are not suitable for identifying the patient. One of the most crucial steps is that the label should be applied to the blood specimen at the patient's bedside and the label should indicate the patient's full name and hospital number. If tubes are prelabeled by the computer systems, the patient identification on the tube must be verified with the patient identification at the bedside. The label on the specimen should also identify the phlebotomist. The blood sample should be checked when it arrives in the blood bank to be sure that all required information is completed. It is not acceptable to change or "correct" information on a specimen label. If there is any doubt about the specimen or the information on the label, a new specimen should be obtained. The blood sample should not be obtained from an arm being used for the infusion of intravenous fluids because these may alter the blood specimen and invalidate the crossmatch. If the blood sample is being obtained from an indwelling catheter, the catheter must be properly irrigated to clear it of any solutions being infused before the blood sample for compatibility testing is collected. One recommended procedure is to flush the line with 5 mL of saline and then withdraw a volume of blood twice the amount that is in the line before obtaining the sample for testing.

13.3 Blood administration sets and filters

Red cells, platelets, granulocytes, fresh frozen plasma, and cryoprecipitate are administered through a filter because fibrin clots and other particulate debris may be present. Traditional blood administration sets have contained filters with a pore size of approximately 170–250 mm, and these effectively remove macroscopic particles. During the 1970s, it was recognized that microaggregates of 20–120 mm composed of platelets, leukocytes, and fibrin strands develop in stored blood [13]. It was believed that an important factor in the development of adult respiratory distress syndrome (ARDS) that often accompanied massive transfusion was the lodging of blood microemboli in the pulmonary microcirculation (see Chapter 14). As the pathophysiology of ARDS was better understood, it became clear that this is a complex situation and that microaggregates are not the primary cause. Clinical studies never established the effectiveness of these microaggregate filters in preventing ARDS [13, 14] and currently they are used only for transfusion of blood salvaged during cardio surgery. The role of filtration has shifted to the increasing use of leukocyte-depleted components.

Initially leukodepletion was done using bedside filters, but indications for blood filtration caused the increasing use of leukodepletion filters (see Chapters 5, 11, and 12]. Leukocyte depletion may reduce the incidence of febrile nonhemolytic transfusion reactions, prevent alloimmunization and the transmission of some cell-associated viruses such as cytomegalovirus, or prevent other transfusion-related immune effects (see Chapter 14). These leukocyte depletion filters remove more than 99% of the leukocytes in the blood component [15], but the filtration process is now usually done in the blood bank soon after the blood is collected. This gives more consistent leukocyte removal and prevents accumulation of cytokines that are produced by leukocytes during blood storage and can cause transfusion reaction (Chapter 14).

Hospitals have policies for the length of time that different types of vascular access devices can be left in place. Some indwelling catheters remain in place for long periods. Intravenous infusion sets using needles for the short-term administration of drugs or blood are usually left in place for only a few hours. This is especially true for infusion sets used to transfuse blood components because the following features increase the potential for problems: small percentage of units of blood components contain some bacteria (see Chapter 14); the administration set is at room temperature, which can facilitate bacterial growth; and fibrin strands and blood cellular debris accumulate on the filter, providing an excellent milieu for bacterial growth. Blood filters will accommodate one to four units of red cells, but substantial debris accumulates usually after two units and the filter is changed then. Since the Food and Drug Administration regulations limit storage of spiked "open" blood products to 4 hours, it is logical to apply this time limit to blood filters. Thus, blood administration sets should be changed after 4 hours, but some hospitals may allow them to be used for up to 24 hours.

13.4 Venous access and the venipuncture

Blood components are administered intravenously. Usually this involves a peripheral vein, but for patients in intensive care units or those receiving many intravenous fluids and blood components over a long period (e.g., hematopoietic cell transplantation (HCTs)), central venous catheters may be used. When using peripheral veins, a vein should be selected that will be large enough to accommodate the needle but is comfortable for the patient. Veins in the antecubital fossa are most accessible and widely used. However, transfusion in these veins limits the patient's ability to flex the elbow during the transfusion. If only one or two units of components are to be given and the expected duration of transfusion is a few hours, the antecubital fossa site is preferred. Veins in the forearm or hand are equally suitable, although venipuncture in these areas may be more difficult or painful for the patient, the veins tend to "roll," and the skin is tougher, sometimes making venipuncture more difficult. For venipuncture using peripheral veins, either steel needles or plastic catheters are used.

The venipuncture should be started before or at the time the blood component is being obtained from the blood bank so that the component can be transfused immediately after it arrives at the patient care unit, thus minimizing the chance of improper storage after leaving the blood bank.

The administration set should be cleared of air before the venipuncture. The venipuncture can be performed with a needle attached to a syringe or attached directly to the blood administration set. Red cells should be administered using a needle or catheter of 19 gauge or larger. Other blood components such as platelets, cryoprecipitate, plasma, and blood derivatives can be administered through smaller needles. Needles as small as 23-gauge thin wall can be used to administer red cells to small patients or adults with small veins. The disadvantage of these small needles for adult patients is the prolonged time required to complete the transfusion. Transfusion of red cells under pressure through these small-lumen needles can cause hemolysis [16], and thus this cannot be used as a method of speeding the transfusion. The flow rate through small-lumen needles can be increased somewhat by diluting the red cells with saline. The transfusion of blood components through a central line that is being used to measure central venous pressure is not recommended. If blood or components are administered through this line, the manometer should be disconnected and the intravenous tubing cleared of the blood components prior to obtaining the central venous pressure reading.

13.5 Infusion solutions

The common use of additive solutions for red cell storage (see Chapter 5) results in red cell components with a hematocrit and viscosity that allows rapid flow rates. Thus, there is no need to add solutions to units of red cells to improve the flow characteristics. This need does sometimes arise for

exchange transfusion or other special transfusion situations. Sodium chloride injection (normal saline) is the solution recommended in the transfusion of blood components containing red cells, platelets, or leukocytes. Other solutions that are satisfactory are 5% normal serum albumin, plasma protein fraction, or ABO-compatible plasma. Certain calcium-free electrolyte solutions can be used; however, these do not have the advantages of plasma or albumin and because they cost more than saline, their use is uncommon. Other solutions can cause hemolysis or red cell clumping and interfere with the success of the transfusion [17, 18]. Five percent dextrose in water is not satisfactory for filling or flushing blood administration sets because red cell clumping and swelling with subsequent hemolysis may occur. In vivo hemolysis of red cells exposed to various intravenous solutions seems to be primarily dependent on the amount of red cell swelling that occurs in vitro [19]. Lactated Ringer's solutions may cause clot formation because they contain calcium, which will recalcify the anticoagulated blood. Other hypotonic sodium chloride solutions also cause red cell swelling and are not recommended.

Medications should not be added directly to the blood component or infused simultaneously through the same intravenous line. Many medications probably are not toxic to blood and could be administered simultaneously, but studies have not been done to establish compatibility and safety. Thus, it is preferable to clamp the blood component intravenous line and clear it with saline prior to administering intravenous medications.

13.6 Identification of the patient and blood component

Ensuring that the correct unit of blood component arrives at the patient care unit in good condition and is administered to the patient begins when the component is released from the blood bank. Blood components may be transported from the blood bank to the patient care unit either by a mechanical transport system or by personnel who hand carry the components. If a mechanical transport system is used, the technologist releasing the blood from the blood bank must ensure that the correct unit is being sent. This involves checking the request to determine that the component being released is what was ordered, reviewing the name, identification number, ABO and Rh type of the recipient and donor unit, the results of the compatibility test, and the appearance of the unit. If the component is to be transported by personnel, the same procedure is followed, but in addition the transport individual usually reviews the identity of the unit and the patient with the blood bank staff member who releases the unit. The blood bank staff member also records the name of the person to whom the component is being released. Each institution has specific policies defining the personnel authorized to receive blood components and transport them to the patient care unit. These should define their responsibilities for identifying the unit and describe proper handling techniques to ensure that the unit is not damaged during transport.

Table 13.2 Steps to check identity of the blood components and the patient.

Patient's chart to determine blood component ordered by physician
Name and ID number of patient's wrist band, transfusion form, and compatibility tag
Patient's ABO and Rh type on patient's medical record, transfusion form, donor blood bag,
    and compatibility tag
Donor unit ID number on bag and compatibility tag
Compatibility tag to determine crossmatch compatibility
Ask patient to state their name

Usually blood is administered by a nurse, but this will be determined by local hospital policy. Often perfusionists administer blood in the operating room. Anyone administering blood should be trained in the procedure. Before beginning the transfusion, it is extremely important to correctly identify the patient and the blood component because this is the last opportunity to detect any clerical errors. Failure to correctly identify the intended blood recipient accounts for about two-thirds of erroneous transfusions [1–4, 8]. In one study, 0.25% of red cell units were transfused to the wrong patient [3]. This is the most common error that results in fatal transfusion reactions [1–4, 8, 9]. It is ideal for two persons to carry out the steps involved in cross-checking the information (Table 13.2).

13.7 Starting the transfusion

All supplies and equipment should be accumulated before initiating the transfusion. The patient should be asked whether there are any questions, and the nurse should attempt to establish an atmosphere of rapport and assurance. The medical record should be checked to determine that the transfusion was ordered and that the correct component is being administered. Baseline vital signs (temperature, pulse, and blood pressure) should be obtained. During storage in the blood bank, the platelet concentrate undergoes continuous gentle agitation. If the platelets are left undisturbed for prolonged periods, the pH may rise and damage may occur. Blood components must not be exposed to hot or cold temperatures, as might occur if they are left in the sunlight or near a cold window. Thus, the transfusion should be started promptly.

Blood components, except platelets and thawed cryoprecipitate, should be stored in a regulated blood bank refrigerator until immediately before transfusion. Because there is an interval between the removal of the red cells from the refrigerator and the initiation of the transfusion, it is important to have policies and procedures to cover this period. Since it is impossible to monitor the temperature of the blood while it is outside the blood bank, it is customary for the blood bank to establish a time limit within which the blood may be out of the control of the blood bank and still be suitable for use. After 45 minutes' exposure to room temperature, the surface temperature of a unit of red cells reaches about 10°C, and after

60 minutes the core temperature is 10°C [20]. The length of time that red cells can be out of the blood bank refrigerator is established by each hospital but is usually between 30 and 60 minutes. Thus, if the transfusion cannot be started within 30 minutes after the blood arrives at the patient care unit, the blood should be returned to the blood bank for further storage. Blood components should not be placed in a refrigerator in the patient care area or near a window, since freezing and thawing or overheating from sunshine may cause red cell hemolysis. Platelets are stored at room temperature, but this does not mean that they can be handled carelessly.

13.8 Rate and duration of transfusion

The rate of transfusion depends on the clinical condition of the patient and the component being transfused. Most patients who are not in congestive heart failure or in danger of fluid overload tolerate the transfusion of one unit of red cells in 1–2 hours. To minimize the severity of a transfusion reaction or the amount of blood hemolyzed if this is going to occur, the first 25–50 mL of the component should be transfused slowly and the patient monitored. If no adverse reaction occurs, the rate can be increased. The transfusion should be completed in less than 4 hours because of the dangers of bacterial proliferation [21], which may occur as the blood warms to room temperature. If premedications were ordered, it should be determined that they have been administered and that ample time has elapsed for them to be effective. In situations requiring transfusion at rates of 100 mL/minute or more, a large-bore (e.g., 8-gauge) catheter should be used.

13.9 Warming of blood

Rapid transfusion of large amounts of cold blood does increase the incidence of cardiac arrest, but it is not necessary to warm blood before transfusion for most patients [22]. Blood warming is recommended in several circumstances (Table 13.3). If blood must be warmed prior to transfusion, this should be done using a blood-warming device designed specifically for blood transfusion [23]. Blood can be warmed in a thermostatically controlled water bath, in a dry heat device with a warming plate, in a heat exchanger with a hot water jacket, or using an in-line microwave device. The choice of the type of device will be based on the setting in which it will be used. Water bath devices are not usually used because they are inconvenient, the unit must be agitated during warming, it cools rapidly after removal from the water bath, and may not be warm when it arrives at the patient bedside. Units warmed in the blood bank and released for patient use cannot be returned to the blood bank for reuse. Modern in-line microwave devices are safe and effective compared with older microwave devices that hemolyzed the red cells [24, 25]. Blood

Table 13.3 Indications and contraindications for warming blood for transfusion.

Indications

Massive transfusions

Trauma situations in which body core-rewarming measures are indicated

Administration rate >50 mL/minute for 30 minutes or more (adults)

Administration rate >15 mL/kg/hour (pediatrics)

Exchange transfusion of a newborn

Patient rewarming phase during cardiopulmonary bypass surgical procedures

Consider blood warming

Potent, high-titered, cold autoantibodies, reactive at body temperature and capable of
    binding complement, thus causing hemolytic anemia in the patient

Raynaud's syndrome or other cold-induced vasoactive effects

Neonatal and pediatric transfusions

Therapeutic apheresis plasma or red cell exchange procedures

Contraindications

Elective transfusions at conventional (slow) infusion rates

Most cold agglutinins encountered in pretransfusion testing

Patient experiencing shivering and discomfort due to the cold (methods to warm the
    patient, not the blood, are indicated)

Platelets, cryoprecipitate, or granulocyte suspensions should not be warmed before
    infusion

Source: Adapted from guidelines for use of blood warming devices; American
Association of Blood Banks.

warming devices usually maintain the temperature at approximately
35–38°C, but always less than 42°C. Most have a thermometer to monitor
the temperature and sound an audible alarm if the temperature is reaching
42°C. Hemolysis may occur when blood is subjected to temperatures
greater than 42°C, although in one study, exposure of blood to 45°C for 30
minutes did not damage the red cells [26]. Blood should never be warmed
by placing it near a radiator, heater, or stove.

13.10 Infusion pumps

Electromechanical pumps that precisely control the flow rate are valuable
for transfusing neonates or small children when the flow rate must be less
than 40 mL/hour or for adults where careful volume control is necessary.
There are two basic types of infusion pumps. One uses a screw mechanism
to advance the plunger on a syringe. This is suitable for transfusion of
small volumes of blood to neonates or very small patients. The second type
uses a peristaltic or roller pump to control blood flow through the tubing
in the administration set. Roller pumps are usually used when it is
desirable to control the flow rates for adult transfusion because they are
not well suited for the very slow flow needed when a small volume is being
transfused. Some pumps require a special tubing set. One consideration
when using either of these pump devices is that if the lumen of the needle
or catheter is small, the pressure caused by the pump can cause hemolysis

[16, 27]. Thus, the rate of flow being controlled by the pump must take into account the lumen size of the needle. Nursing staff should be trained in the proper use of infusion pumps.

A separate method of providing pressure to speed the rate of transfusion is to place the blood container inside a pressure bag or pressure duff specifically designed for this purpose. The pressure bag is inflated, causing pressure against the blood container and increasing the flow out of the blood container. This is simple and effective but has the drawback of poor control of the exact amount of pressure being applied. Excessive pressure can cause rupture of the blood container or hemolysis if the lumen of the needle is inadequate for the amount of pressure being applied. If these devices are to be used, the blood should be transfused through a needle or catheter with a lumen of at least 18 gauge [28].

13.11 Nursing care of patients receiving a transfusion

Effective nursing care is important for patients receiving transfusions of all blood components, including platelets, plasma products, cryoprecipitate, and albumin, as well as red cells. The nurse can reduce the patient's anxiety by answering any remaining questions and establishing rapport with the patient. If premedication is to be given, this should be done and adequate time allowed for the medication to take effect. All supplies and equipment should be accumulated so that starting the transfusion can begin efficiently, thus also adding reassurance to the patient. During the first 15 minutes, the rate of transfusion should be slow: approximately 2–4 mL/minute. This will minimize the volume transfused if the patient experiences an immediate reaction. The nurse should observe the patient during at least the first 5 minutes of the transfusion, then return after 15 minutes to ensure that the transfusion is proceeding uneventfully. If so, the rate of flow can be increased to that ordered by the physician. Baseline values for temperature, pulse, respirations, and blood pressures should be obtained before beginning the transfusion and should be determined every hour until 1 hour posttransfusion. Failure to record vital signs is the most frequent variance from blood transfusion protocols [29]. At the completion of the transfusion, the nurse should record whether any adverse reaction occurred.

13.12 Transfusion techniques for children and neonates

The selection of components for and special transfusion needs of children and neonates are described in Chapter 12. Issues related to obtaining informed consent, obtaining the pretransfusion blood sample, identifying the patient and the component, delivering infusion solutions, starting the transfusion, and administering nursing care during the transfusion are

similar to those described above for adults. However, because of their size, neonatal patients and small children require special attention to the methods of administering the transfusion.

Transfusion in small patients is usually accomplished using needles or catheters ranging in size from 22 to 27 gauge. Hemolysis can occur when red cells with a high hematocrit or longer storage time are forced by pressure through small-bore needles. There is no exact limit that can be specified for each possible combination of needle size, flow rate, hematocrit, and blood storage time. Thus, it is advisable to use the largest size needle appropriate for the patient, to use red cells with a hematocrit of about 60%, which is the usual hematocrit of cells suspended in additive solutions, and to administer the red cells slowly, usually less than 10 mL/hour for neonates. The older the red cells, the more important each of these factors becomes. The use of infusion pumps is described above.

A relatively large amount of blood in relation to the volume transfused may be used to fill the "dead space" of the tubing sets. Pediatric sets and smaller filters are available to reduce the amount of blood required to fill this space. One concern has been that microaggregates that form in stored blood might be a particular problem for neonates because they could pass into the systemic circulation via a patent foramen ovale or intrapulmonary shunting. Usually neonates are transfused with blood that has not been stored for long periods, and thus does not contain many microaggregates. Also, most red cells used for neonates are leukodepleted and, thus, free of microaggregates.

When a small volume of blood component is being transfused, this can be measured using a buret in the tubing set. Some sets are available that are designed specifically for neonates and contain a buret, a small-volume filter, and short tubing to minimize the volume of component necessary for the dead space. For neonates the volume of component being administered is usually small, and the red cells may be contained in a syringe rather than the usual plastic bag. Infusion using a syringe placed in an electromechanical delivery pump allows accurate control of the rate of infusion of small volumes and does not cause hemolysis [27, 30].

Because the volume of blood being transfused to small patients is also small, warming the blood is usually not unnecessary. However, the relationship of the volume being transfused to the patient's total blood volume and the rate of transfusion must be considered. However, even for small volumes of blood, warming may be necessary if the patient is small or infusion rate is rapid. Many of the standard blood warming devices have adaptors or small inserts that reduce the volume necessary to fill the device.

13.13 Transfusion of platelets and plasma

The general procedures described in this chapter should be followed when platelets or plasma are being transfused. Although they do not contain red cells, hemolysis can occur from antibodies contained in the platelets or plasma and other serious, even fatal, reactions also occur (see Chapter 14).

Because platelets and plasma require unique storage conditions that are best provided in the blood bank, they should be transfused promptly after being released from the blood bank. Cellular aggregates or fibrin strands may be present and so platelets and plasma should be transfused through a blood filter. Filters cause very little loss of platelets [31, 32] and no loss of platelet aggregation [33]. If the volume of the blood administration set is large, it can be flushed with saline so that all the component is given to the patient. Platelets will have a volume of approximately 250 mL. They can be transfused as rapidly as the patient will tolerate, usually in about 1 hour but not more than 4 hours. The nursing care is the same as for a red cell transfusion.

13.14 Transfusion of hematopoietic stem cell products

When marrow, peripheral blood stem cells, or umbilical cord blood are used for transplantation, they are actually given as an intravenous transfusion. Most of the key steps in the transfusion of blood components also apply to transfusion of hematopoietic stem cells (Table 13.4) [34]. Even the most basic steps such as obtaining the physician's written order and properly identifying the product and the patient are essential. This will ensure that the correct stem cell product is sent from the laboratory and given to the correct patient. There is a tendency to believe that everyone knows what is to be done because of the small number of patients receiving a stem cell transfusion at any time. This must be avoided and all steps followed carefully to avoid errors. The patient identification information must be consistent with the information on the bag of cells and attached documents or tags in order to avoid a catastrophic error.

Patients receiving HCTs often experience side effects during the transfusion [34,35] that may be unique to these products because of the cellular content and/or the presence of liquid or cryopreservative solutions. These should be anticipated and the patient premedicated if indicated. Side effects range from mild chills, fever, flushing, nausea to (rarely)

Table 13.4 Key steps in transfusing hematopoietic stem cells.

Obtain physician written order
Anticipate potential reactions or toxicities and plan nursing care accordingly
Carry out proper transport and handling of product from laboratory to patient bedside
Plan for appropriate thawing or other product preparation for transfusion
Premedicate the patient, if indicated
Identify the patient and stem cell product by checking unit numbers
Select proper venous access
Select proper infusion set including filter, if indicated
Obtain pretransfusion vital signs
Determine infusion rate
Monitor patient during infusion
Complete transfusion record and report adverse reactions to laboratory

life-threatening cardiac, pulmonary, renal, neurologic, or anaphylactic reactions [34].

The HCT product must be stored properly while being transported to the bedside. It has been believed for many years that thawing must be rapid [34] to avoid formation of free water, which could damage the cells as the ice crystals melt. In addition, dimethyl sulfoxide (DMSO), used as a cryopreservative, has been thought to be toxic to stem cells upon prolonged exposure, and thus transfusion should be accomplished as soon as possible after thawing [34]. Traditionally, the stem cells have been thawed in a 37°C water bath in the patient's room and transfused immediately, although apparently may be in contact with 10% DMSO for up to 1 hour [36, 37]. Some transplant centers thaw and wash away the DMSO in the cell therapy laboratory to reduce the reactions associated with DMSO transfusion [38]. Such a washing step is usually used for umbilical cord blood (see Chapter 18) [39]. As for other blood components, HCTs should be taken to the bedside and infused promptly and not stored in refrigerators, or left on countertops or window sills in patient care areas as the cells may be damaged. Frozen products can be transported on dry ice. Usually frozen products are thawed in a 37°C water bath at the bedside and the transfusion began immediately.

Hematopoietic progenitor cells (HPCs) are transfused intravenously through a central venous line. The 10% DMSO solution usually used for cryopreservation is irritating to smaller veins. The HPC products are usually transfused through a standard 170-m blood filter to trap any large cell clumps, but the presence of many visible clumps may indicate a problem that requires rapid investigation. HPCs are infused rather rapidly, ranging from 5 to 50 mL/minute, although it appears that slower rates reduce the severity of reactions [34] but leave the stem cells in contact with DMSO longer.

The patient should be monitored, and nursing care is similar to that for a red cell transfusion. Vital signs are obtained before HCT transfusion and about every 15 minutes during infusion. Because of the uniqueness of the cells being infused and the potential for serious side effects, nursing or medical personnel should be in constant attendance during the transfusion. Noncryopreserved products are usually transported to the bedside at room temperature but should be administered promptly as with the other blood products. Because they do not contain cryopreservatives, transfusion reactions are fewer and more mild than from cryopreserved products [35]. Since these products have not been cryopreserved, their red cell content may be substantial. This must be considered if the patient and donor are ABO incompatible in order to avoid a hemolytic transfusion reaction. Hemolysis can occur from either incompatible red cells (patient O; donor A) or incompatible plasma (patient A; donor O). The HPC product can be modified to prevent this (see Chapter 18), but the personnel administering the transfusion of HPCs must be aware of this possibility and check the patient and donor records to prevent hemolysis.

The transfusion should be documented in the patient's record and a transfusion record returned to the laboratory where it is used for quality improvement [40].

13.15 Transfusion in the nonhospital setting

Program rationale

The increasing emphasis on health care cost reduction is shifting more health care into the nonhospital setting. As a part of this shift, some transfusions are provided in the nonhospital setting [41, 42]. Additional impetus for transfusion in the nonhospital setting is the growing population of elderly patients and those with chronic debilitating diseases. In many situations, the nonhospital setting is a major clinic that functions almost as part of the hospital. However, there are many other situations in which the possibility of providing out-of-hospital transfusions is considered. These include individual physicians' offices or clinics not part of a hospital, the patient's home, or a nursing or long-term care facility. Provision of transfusions in a nonhealth care facility, such as the patient's home, where other experienced health care personnel, equipment, and care systems are not available is a particularly complex situation and this practice is not widely used. Patients with hemophilia A have been receiving home therapy with coagulation factor concentrates for years. This has proven to be safe and extremely effective. Transfusion of other blood components such as red cells, platelets, or plasma carries additional risks. This may provide a convenient service for patients with end-stage malignancy; acquired immune deficiency syndrome; inflammatory, collagen vascular, neuromuscular, or other debilitating diseases; hemoglobinopathies; end-stage renal disease; myelodysplasia; chronic low-grade bleeding; or chronic hemolysis. Patients should be stable, not acutely ill, and with satisfactory cardiovascular and respiratory function and fluid balance. Thoughtful discussion of the pertinent issues have been developed [41, 42].

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14 Complications of Transfusion

The long-term mortality of patients following transfusion is 31% in the first year, 14% in the second year, and 10% per year in years 3–5 following transfusion [1]. In a study of the long-term survival of patients who received transfusions, 46.9% of 2899 patients who received 10,760 units of red cells were alive at 5 years following transplant. The patients who survived for at least 5 years represented 41% of the transfused RBC units and 36% of transfused fresh frozen plasma units. Shorter patient survival was associated with increasing patient age, increasing number of RBC units transfused, receipt of plasma or platelets [2]. While most is due to underlying disease, the complications of transfusion are serious, even life-threatening. The complications of transfusion can be categorized as immunologic and nonimmunologic (Table 14.1). The immunologic complications involve various forms of what are usually thought of as transfusion reactions, but more recently there is an increased interest in the immunomodulation effects of transfusion. Nonimmunologic complications are usually caused by the physical effects of the blood component or the transmission of disease. Many of the complications of transfusion are caused by the leukocytes contained in the blood components (Table 14.2). This chapter considers all of these complications except transmission of disease, which is discussed in Chapter 15.

Adverse effects during or shortly after completion of the transfusion occur after about 1–3% of transfusions [3]. The incidence of long term or later adverse effects of transfusion are more variable because many of these are the result of disease transmission, the likelihood of which depends on the prevalence of the diseases in the donor population, the natural history of the patient's basic disease, and the extent of followup care the patient receives. It has been estimated that almost 20% of transfusions result in some kind of adverse effect, with about 0.5% of these considered serious [3, 4]. The short-term fatality rate following transfusion is about 1–1.2 per 100,000 patients who receive a transfusion [5–7]. This amounts to approximately 35 transfusion-related deaths per year in the United States, although fatalities reported to the Food and Drug Administration (FDA) range from 54 to 73 from 2006 to 2009 [8]. The remarkable progress in reducing posttransfusion infection has not been matched with progress to reduce noninfectious serious hazards of transfusion (NISHOT) [9–12]. The most common NISHOT are hemolytic transfusion reactions (26%), transfusion-related acute lung injury (TRALI) (48%), circulatory

Table 14.1 Complications of transfusion.

Immunologic complcations	
Immunologic transfusion reactions	
Red cell hemolysis	Antibodies in patient or donor
Febrile	White blood cells in component
Transfusion-related acute lung injury	White blood cells or cytokines in components
Allergic	Plasma proteins in components
Anaphylactic	Plasma proteins (IgA) in component
Graft-versus-host disease	Caused by viable lymphocytes
Immunization or immune modulation	
White cells	Febrile reaction; platelet refractoriness
Platelets	Febrile reaction; platelet refractoriness
Red cells	Hemolytic reaction
Graft acceptance	Caused by white cells
Cancer recurrence	Caused by white cells
Postoperative infection	Caused by white cells
Nonimmunologic complications (see Chapter 15)	
Disease transmission	
Viral	Caused by contaminated component
Bacterial	Caused by contaminated component
Parasitic	Caused by contaminated component
Other adverse effects	
Circulatory overload	Caused by whole blood
Citrate toxicity	Caused by citrate anticoagulant
Bleeding tendency	Massive transfusion
Electrolyte imbalance	May cause arrhythmia
Hemosiderosis	Caused by chronic transfusions
Embolism	Air or particles
Cold blood	May cause arrhythmia

overload (11%), transfusion-induced graft-versus-host disease (GVHD), and metabolic alterations [5, 6, 8, 9]. Adverse effects due to NISHOT may be 100–1000 times more common than transfusion-transmitted infections [9].

Table 14.2 Adverse effects of leukocytes in blood components.

Immunologic effects
Alloimmunization
Febrile nonhemolytic transfusion reactions
Refractoriness to platelet transfusion
Rejection of transplanted organs
Graft-versus-host disease
Transfusion-related acute lung injury
Immunomodulation
Increased bacterial infections
Increased recurrence of malignancy
Infectious disease
Cytomegalovirus
HTLV-I
Epstein–Barr virus

Since 1996, the United Kingdom has operated a hemovigilance system called serious hazards of transfusion [4]. In addition to complications of transfusion, the system tracks handling and storage errors. From 1996 to 2009, 6653 complications have been reported [4] (Table 14.3) associated with transfusion of 31,788,155 units of red cells, platelets, plasma, and cryoprecipitate. This is probably the most comprehensive data available, although the French have implemented an active hemovigilance system in which the outcome of every transfusion is recorded. Hemovigilance systems are being established in many countries including the United States.

14.1 Immunologic complications of transfusion resulting in transfusion reactions

Hemolytic transfusion reactions

A variety of settings can lead to red cell hemolysis in transfusion recipients (Table 14.4). The most dangerous immunologic complication of transfusion is an ABO-incompatible hemolytic transfusion reaction. Twenty six percent of transfusion fatalities are due to hemolytic reactions and 16% of transfusion fatalities reported to the US FDA are caused by ABO-incompatible transfusions [8]. From 3 to 19 patients per year die from fatal ABO-incompatible transfusions [8], giving an apparent incidence of 1 in 300,000 patients transfused.

ABO-incompatible hemolytic transfusion reactions are very dangerous because the patient has preformed ABO antibodies that often are IgM and bind complement, causing activation of the complement system with associated systemic manifestations and leading to red cell lysis. However, the nature and severity of the symptoms do not correlate with the severity or ultimate outcome of a hemolytic transfusion reaction [13–15]. In general, the occurrence of symptoms relates to the volume of incompatible red cells received −64% after >50 mL and 16% from <50 mL [14]. However, some patients may tolerate an entire unit with no unusual signs or symptoms. The reaction may begin almost immediately upon beginning the transfusion or up to several hours after transfusion. The most common signs and symptoms that may accompany a hemolytic transfusion reaction are fever and chills.

Although usually IgM red cell antibodies activate complement and IgG antibodies do not, the signs and symptoms of a hemolytic transfusion reaction to either type of antibody may be similar. For IgM-type antibody–antigen reactions, the pathophysiology may result from the activation of complement system, especially C3a and C5a, which are vasoactive and cause the release of serotonin and histamine from mast cells, possibly accounting for the hypotension that often occurs [16]. The symptoms of IgG-type hemolysis are probably due to several cytokines [17]. Increases in IL-1, IL-6, IL-8, and tumor necrosis factor (TNF) occur in an in vitro system of IgG-mediated hemolysis. IL-1 may be a key mediator, since it causes fever, neutrophil and endothelial cell activation,

Table 14.3 Adverse events reported through the United Kingdom SHOT hemovigilance System[a].

	Total	Incorrect blood component transfused	I & U	Handling & storage	Anti-D related events[b]	Acute transfusion reaction	Hemolytic transfusion reaction	TRALI	Transfusion-associated circulatory overload	Transfusion-associated dyspnea	PTP	TA-GvHD	Transfusion-transmitted infection	Autologous transfusion
Death in which transfusion reaction was causal or contributory	138	27	4	0	0	19	11	42	5	0	2	13	15	0
Major morbidity probably or definitively attributed to transfusion reaction (imputability 2/3)	495	116	3	0	25	58	48	165	18	1	13	0	48	0
Minor or no morbidity as a result of transfusion reaction	5998	3439	161	335	361	1154	383	50	29	4	34	0	6	42
Outcome unknown	15	11	0	0	0	3	1	0	0	0	0	0	0	0
Total[c]	6646	3593	168	335	386	1234	443	257	52	5	49	13	69	42

[a] See Cohen H and the Steering Group. Serious hazards of transmission annual report, 2009. UK National Health Service.
[b] Total excludes 7 cases from 1998–1999 that were not classified.
[c] Cases with potential for major morbidity included in the Anti-D data are excluded from this table.

Table 14.4 Causes of red cell hemolysis associated with red cell transfusions.

Red cell antibody in recipient
Red cell antibody in transfused plasma
Large volumes of aged red cells
Addition of drugs or intravenous solutions to donor unit
Bacterial contamination of red cell unit
Red cell enzyme deficiency in the donor
Excessive warming of donor unit
Erroneous freezing of donor unit
Trauma to red cells from extracorporeal instruments

Source: Reproduced with permission from McCullough J. Transfusion medicine. In: Handin RI, Lux SE, Stossel TP, eds. Blood: Principles and Practice of Hematology. Philadelphia: Lippincott, Williams and Wilkins, 2003, p. 2053.

and increased expression of IL-1 itself, IL-8, IL-6, TNF, complement, tissue factor, and plasminogen activator inhibitor [17, 18].

Several investigators have shown that cytokines also play a key role in hemolytic transfusion reactions. IL-8, which activates neutrophils, is increased in an in vitro ABO hemolysis assay [19], as is TNF [20]. IL6 and monocyte chemo attractant protein 1 [21]. Thus, it appears that cytokine storm occurs in response to red cell incompatibility. This could be somewhat like a final common pathway for both the IgM- and IgG-incompatible systems. Another common feature is that the cytokine production appears to be lacking if complement is inactivated. Thus, binding of complement to the red cell surface is probably a major factor in initiating cytokine production.

Coagulopathy is also often part of a hemolytic transfusion reaction, especially those due to IgM antibodies. The coagulation system may be activated in several ways: antigen–antibody complexes activate Hageman factor (XII), which in turn activates the coagulation system; red cell stroma contain thromboplastic substances that activate the coagulation system; activation of platelets releases platelet factor 3, which activates the coagulation system; hypotension leads to tissue hypoxia, causing release of tissue factors, which in turn activates the coagulation system. Thus, through one or more of these mechanisms, patients with a severe hemolytic transfusion reaction may develop a coagulopathy and/or disseminated intravascular coagulation. Cytokines may also have a role in coagulopathy associated with hemolytic transfusion reactions.

Procoagulant activity is induced in monocytes by endotoxin, TNF, and C3. In an in vitro assay of ABO incompatibility, procoagulant activity was generated and it was hypothesized that this could contribute to the disseminated intravascular coagulopathy (DIC) that develops with ABO-incompatible hemolysis [22].

Hemolytic transfusion reactions may also cause oliguria and renal failure. At least two mechanisms may be involved. These are (a) activated Hageman factor (XII) that promotes the release of bradykinin, which causes vasoconstriction and hypotension, and (b) DIC that causes the
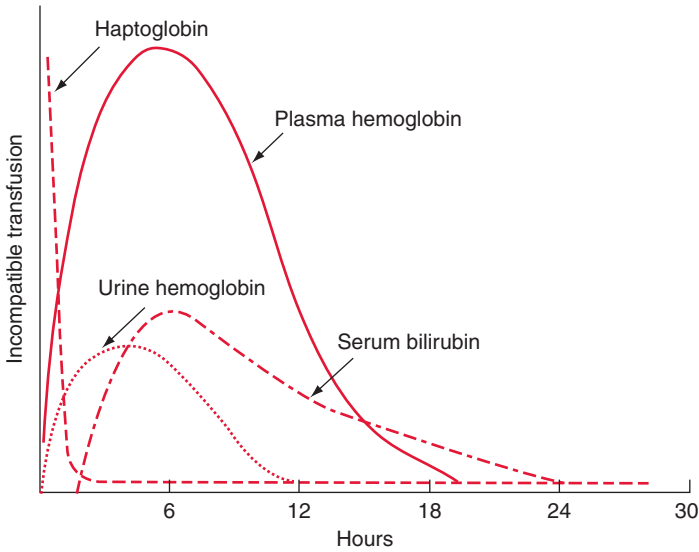
Figure 14.1 Time relationship of pigmentary changes in serum following hemolytic transfusion reaction (not to scale). (Reproduced from Huestis DW, Bove JR, Case J, eds. Complications of transfusion. Practical Blood Transfusion, 4th ed. Boston: Little, Brown and Company, 1988, p. 251.)

formation of microthrombi. These events in combination cause a reduced renal blood flow and renal damage.

There is a classic pattern of alteration in laboratory tests in a hemolytic transfusion reaction (Figure 14.1). In any series of cases, the incidence of abnormalities in different tests cannot be easily related to this pattern because patients will be studied at different periods after the onset of hemolysis. Nevertheless, one study illustrates the kind of laboratory abnormalities seen in practice. Pineda et al. [13] found that in most patients who experienced an acute hemolytic transfusion reaction there was hemoglobinemia and/or hemoglobinuria, reduced serum haptoglobin, positive direct antiglobulin test, elevated bilirubin, and unexpected red cell antibody (Table 14.5). Thus, in general, laboratory testing should be quite

Table 14.5 Laboratory test abnormalities in patients experiencing a hemolytic transfusion reaction.

Hemoglobinemia or hemoglobinuria	87%
Positive direct antiglobulin test	87%
Reduced haptoglobin	87%
Unexpected red cell antibody	85%
Elevated bilirubin	80%
Urine hemosiderin	49%
Methemalbumin	0%

Source: Pineda AA, Brzica SM, Taswell HF. Hemolytic transfusion reaction: recent experience in a large blood bank. Mayo Clin Proc 1978; 53:378.

helpful in diagnosing hemolysis, and the red cell serologic studies have a high likelihood of detecting the antibody involved.

14.2 Delayed hemolytic transfusion reaction

A delayed hemolytic transfusion reaction (DHTR) occurs in a patient in whom no red cell antibody was detected at the time of compatibility testing but who experiences accelerated destruction of the transfused red cells after an interval during which an antibody develops to the transfused red cells. The interval after transfusion may be as little as 24 hours or up to about 1 week. A DHTR may be symptomatic or asymptomatic. Most DHTRs are identified by a decrease in hemoglobin after transfusion [23]. Other signs or symptoms of a hemolytic transfusion reaction such as fever, elevated bilirubin, or reduced urine output can occur, but these are not common. The DHTR can also be recognized by finding a red cell antibody in a subsequent blood specimen and initiating a serologic investigation that reveals a positive direct antiglobulin test and the red cell antibody in an eluate of the patient's red cells. This situation has been called a delayed serologic transfusion reaction (DSTR) to distinguish it from a situation in which there is a clinical effect of the process. A DHTR or DSTR occurs about once in 1900—3000 units transfused [23, 24]. The DSTRs occur more frequently than DHTRs at a ratio of about 2:1 [23]. A DHTR can create substantial clinical difficulties because the most common sign—falling hemoglobin—can imply other important problems. For instance, a search for occult bleeding might be undertaken or the DHTR can appear as a sickle crisis [25], cause renal failure [26], or masquerade as autoimmune hemolytic anemia [27].

Because of the time involved for antibody formation in primary immunization, DHTRs usually occur in women (immunized during a previous pregnancy or transfusion) or previously transfused males. The antibodies involved are most commonly Rh such as E or c, but also Kell, Fy^a, Jk^a, and many others [23]. The sensitivity of the antibody screening test in the patient is very important in preventing DHTRs [28], since an insensitive method will miss weak antibodies. Thus, the best strategy for preventing a DHTR is use of an effective antibody screening method. Treatment of a DHTR depends on the severity of the symptoms. A falling hemoglobin level can be managed by transfusion of red cells negative for the offending antigen. More severe symptoms of hemolysis are treated as for any hemolytic transfusion reaction even though they may be occurring several days after the transfusion.

14.3 Hemolysis due to passenger lymphocyte syndrome

When some organs, especially liver, are transplanted, large numbers of (passenger) lymphocytes may be carried along [29]. Some

immunosuppressive regimens are B-cell sparing, allowing these donor passenger lymphocytes to remain viable and functional. When blood group incompatibility, especially ABO, exists, the donor-derived passenger lymphocytes may produce antibodies that react with the recipient's red cells creating mild to severe hemolysis [30, 31]. Although ABO is most commonly involved, several antibody specificities have been observed [31, 32]. Passenger lymphocyte-related hemolysis has occurred following transplantation of liver, lung, heart, kidney, pancreas, and intestine [33]. Hemolysis usually develops within 2 weeks and is mild to moderate, but can be severe and antibodies may persist up to a year [32]. If hemolysis is severe, donor type red cells can be used or plasma or red cell exchange carried out (see also Chapter 12).

14.4 Nonimmunologic hemolysis mimicking a transfusion reaction

Signs and symptoms of hemolysis can occur following transfusion due to transfusion of hemolyzed blood or patient manipulations. If the red cells are not stored properly and exposed to extremely high or low temperatures, or if they are mixed with inappropriate solutions or transfused under pressure through a small bore needle (see Chapter 13), they may be hemolyzed. This may appear to be an immunologic hemolytic transfusion when in fact it is transfusion of free hemoglobin. Intravascular manipulations within the patient can also appear to be an immunologic hemolytic transfusion reaction. Percutaneous mechanical thrombectomy uses devices to clear intravascular thrombi by a combination of mechanical dissolution, fragmentation, and aspiration. A moderate transient increase in plasma hemoglobin usually occurs during the procedure [34–36], but this hemolysis can be severe and mimic an immunologic hemolytic transfusion reaction [37].

14.5 Febrile nonhemolytic transfusion reactions

Febrile nonhemolytic transfusion (FNHT) reactions occur in association with about 0.5–1.0% of transfusions. It has been believed that they are caused by leukocyte antibodies present in the patients that react with leukocytes present in the transfused components [38]. The severity of the reaction is directly related to the number of leukocytes in the blood component [39]. However, many patients who experience an FNHT reaction do not possess leukocyte antibodies, and the presence of a leukocyte antibody does not always predict a reaction [40–43]. Patients' sera may contain HLA antibodies or antibodies to granulocytes or platelets [41, 44]. Thus, although leukocyte antibodies may play a key role in FNHT reactions, other factors such as cytokines are also involved (see below).

These leukocyte reactions do not cause red blood cell hemolysis but can be extremely uncomfortable for the patient and are potentially fatal.

Symptoms such as chills, fever, headache, malaise, nausea, vomiting, and chest or back pain may persist for up to 8 hours [38, 43] and seem to be caused by immune damage to donor granulocytes. Thus, it is not surprising that these febrile/leukocyte reactions can be prevented by removing leukocytes from the blood components [38, 40, 41, 43]. The increasing use of leukocyte-depleted blood components for a variety of conditions (see Chapters 11 and 12) is reducing the incidence of FNHT [45–47]. Acetaminophen and dephenhydramine have become commonly used to prevent FNHT reactions, but it is not clear whether this is helpful and no sound data exist to support this practice [48].

14.6 Allergic reactions

Allergic reactions are probably the most frequent kind of reaction, occurring after 1–2% of transfusions. They range from harmless but annoying (hives) to severe respiratory or anaphylactic. As many as 30% of donors under age 30 have allergic disorders [49], and IgE antibodies probably are involved in many allergic reactions [50]. However, there are no practical laboratory tests or medical history questions that would be effective donor screening techniques to prevent allergic reactions [49]. Histamine may be involved because levels increase during storage of red cell units containing leukocytes [51]. Allergic reactions are more likely to occur from red cells that have been stored longer [51]. Plasma histamine levels are higher in patients experiencing anaphylactoid reactions compared with other types of reactions [51]. These data and those described below for reactions to platelet transfusions suggest that leukocytes in stored blood components produce cytokines and vasoactive substances that play a key role in transfusion reactions. Delayed hypersensitivity can be passively transferred to a patient from donor plasma [52], although current use of additive solutions for red cell storage makes this unlikely at present.

Allergic reactions involving hives only with no other symptoms are the only situation in which the patient can be given an antihistamine, and after 15–30 minutes the transfusion can be restarted. It may be that as more is learned about the role of IgE antibodies and cytokines in transfusion reactions, these reactions will be better understood.

14.7 Pulmonary reactions—transfusion-related acute lung injury

Acute lung injury (ALI) following transfusion is more prevalent than previously believed [53, 54]. Posttransfusion ALI is associated with female donor plasma, number of pregnancies in donors of plasma, presence of antigranulocyte, or HLA class II antibodies in donor plasma [53, 54]. A very severe type of transfusion reaction is the acute, sometimes fatal,

Table 14.6 Recommended criteria for TRALI and possible TRALI.

1. TRALI criteria
    (a) ALI
        (i) Acute onset
        (ii) Hypoxemia
            $PaO_2/FiO_2 \leq 300$ or SpO_2 <90% on room air or other clinical evidence of Hypoxemia
        (iii) Bilateral infiltrates on frontal chest radiograph
        (iv) No evidence of left atrial hypertension (i.e., circulatory overload)
    (b) No preexisting ALI
    (c) During or within 6 hr of transfusion
    (d) No tetmporal relationship to an alaternative risk factor for ALI
2. Possible TRALI
    (a) ALI
    (b) No preexisting ALI
    (c) During or within 6 hr of transfusion
    (d) A clear temporal relationship to an alternative risk factor for ALI

Source: Kleinman S, Caulfield T, Chan P, et al. Toward an understanding of transfusion-related acute lung injury: statement of a consensus panel. Transfusion 2004; 44:1774–1789.
Acute lung injury, ALI.

pulmonary reaction that has been termed TRALI [55–59]. This is the leading cause of transfusion fatalities [8]. Thus, plasma-containing components are usually the cause, although TRALI may occur following red cell transfusion [8]. TRALI begins within 4 hours of initiating the transfusion and consists of fever, hypotension, tachypnea, and dyspnea, with diffuse pulmonary infiltrates on X-ray and the general clinical presentation of noncardiogenic pulmonary edema [53, 54, 60, 61]. TRALI was thought to occur once in 4500 transfusions [55, 56], but it may be more common than this because of underreporting and failure to recognize more mild cases [58, 59].

Pulmonary reactions following transfusion can range from mild to severe [53, 54]. Thus, it is important to have a clear definition of TRALI. A consensus conference [62] recommended criteria for the diagnosis of TRALI (Table 14.6)

Leukocyte antibodies have been thought to be the inciting cause of TRALI, as they are found in almost 90% of cases [55, 63]. Many of the leukocyte antibodies have been HLA but some have been granulocyte-specific [54, 64]. This was first described in 1957, when Brittingham reported that transfusion of 50 mL of whole blood containing a leukoagglutinating antibody caused fever, vomiting, diarrhea, chills, dyspnea, tachycardia, hypotension, cyanosis, pulmonary infiltrates, and leucopenia [38]. Most TRALI type reactions seem to involve transfusion of leukocyte antibodies in the donor unit that react with the patient's leukocytes, but TRALI due to intradonor incompatibility has been reported [65]. Two different mechanisms for TRALI have been proposed and seem to make sense. The first is an antibody-mediated event involving

the transfusion of antigranulocyte or HLA class I or class II antibodies into patients whose leukocytes contain the cognate antigens. This activates complement, causing adherence of granulocytes to pulmonary endothelium with release of proteolytic enzymes and toxic oxygen metabolites, which cause further endothelial damage. However, leukocyte antibodies are not present in all cases of TRALI, and the variation in the type of antibody and its presence in either the donor or recipient suggest that there are other factors involved. The second mechanism is a two event process in which the first event is a clinical condition of the patient leading to pulmonary endothelial activation with the second event being the transfusion of plasma containing antigranulocyte or anti-HLA antibodies or other biologic response modifiers such as lipids [64]. At the time of TRALI, lipids with neutrophil priming activity are increased [66] and that two events are necessary for TRALI to occur: first a phenomenon causing general adherence of neutrophils to pulmonary endothelium, then neutrophil activation leading to pulmonary endothelial damage. The initial adherence to endothelium is caused by the underlying disease process, and then the neutrophil-priming lipids in the transfused blood component cause activation and endothelial damage [66]. In some patients, transfused leukocyte antibodies could also participate in this process. At present, methods for leukocyte antibody screening of donors are being developed but is not done routinely. Instead blood from multiparous or previously transfused donors is avoided, and most plasma-containing components are now produced with male donor plasma. This has been quite successful in reducing TRALI [67–69].

14.8 Anaphylactic reactions

Anaphylactic reactions to transfusion may be due to antibodies against IgA, complement C4, haptoglobin, or other unknown plasma proteins. Patients who are IgA deficient and have anti-IgA antibodies may experience an anaphylactic reaction if they receive blood components containing IgA [70, 71]. These reactions probably occur 1 in 20,000 to 1 in 47,000 transfusions [71] and only in the rare patients who are severely IgA deficient and not the many who lack only an IgA subclass. IgA deficiency ranges from 1 in 223 to 1 in 3000 blood donors [71, 72]. The much lower incidence of anaphylactic reactions suggests that most of these IgA-deficient individuals will not have an anaphylactic transfusion reaction. Anti-IgA titers are not predictive of the likelihood of a reaction [72]. These reactions may be dramatic and rapidly fatal. The treatment is the same as for any anaphylactic reaction. The reactions can be prevented by using red cells or platelet concentrates washed to remove plasma IgA and by using plasma components prepared from IgA-deficient donors. Most blood banks have access to blood components from IgA-deficient donors through rare donor registries. Many community blood centers have screened donors for IgA and have a small registry of local IgA-deficient donors.

C4a and C4b complement fragments carry the Chido and Rogers determinants that can be detected by red cell serologic methods (see Chapter 9). Patients with these antibodies may exhibit variable anaphlactoid reactions following transfusion with plasma products.

Patients who lack haptoglobin due to gene deletion may form haptoglobin antibodies and when exposed to plasma products experience severe anaphylactic reactions [73, 74]. Some anaphylactoid reactions may be related to cytokines in stored blood components (see below).

14.9 Hypotensive reactions

Occasionally, severe hypotensive reactions have been reported to occur following transfusion of patients taking angiotensin-converting enzyme inhibitors who receive their transfusion through a bedside leukodepletion filter [75–77, 5]. These reactions are the result of bradykinin that is not inactivated in patients taking angiotensin-converting enzyme inhibitors for hypertension. The phenomenon can occur with both red cell and platelet transfusions and occurs rarely since prestorage leukodepletion has become more widely used.

14.10 Reactions to platelet transfusions

Patients with platelet or HLA antibodies may have febrile nonhemolytic reactions, probably caused by leukocytes contained in the platelet concentrates. The reactions usually involve chills and fever, but platelets may be trapped in the pulmonary capillaries, causing dyspnea and pulmonary edema. These febrile nonhemolytic reactions occur following about 5% of platelet transfusions. The reactions were thought to be caused by antibodies to leukocytes and were considered similar to febrile nonhemolytic reactions from units of red cells. However, the reactions sometimes occur in nontransfused males and are more common when stored platelets are used [78] suggesting that some bioactive substance other than leukocyte antibodies might be involved. Heddle et al. [78] demonstrated that when platelet concentrates were separated into the plasma and the platelets, the plasma component was more likely than the platelets to cause a reaction. The reactions were correlated with the concentrations of IL-1 and IL-6 in the plasma, and the concentrations of these cytokines increased during storage of the platelets. Thus, this landmark study established that many (if not most) reactions to platelet transfusion are the result of cytokines, not antigen–antibody reactions. These authors subsequently demonstrated that removal of the leukocytes soon after collection of the blood prevented the accumulation of cytokines in the platelet concentrate and avoided platelet transfusion reactions [79]. Removal of leukocytes at the time of transfusion does not decrease the incidence of platelet transfusion reactions [80].

Allergic reactions may also occur with platelet transfusions. In one study, the IL-6 levels in platelet concentrates were correlated with allergic reactions [81], suggesting that cytokines might be involved in allergic as well as febrile reactions.

14.11 Reactions to granulocyte transfusions

Transfusion reactions are common following granulocyte transfusions [82]. In some series, almost all transfusions were associated with fever and chills—some so severe that meperidine was used to control the rigors. Many of these reactions are probably caused by the physiologic activity of the large dose of leukocytes being administered to infected neutropenic patients. Some of the reactions are also probably attributable to leukocyte antibodies that form commonly in these patients (see below). Since granulocyte concentrates do not undergo a leukocyte crossmatch, leukocyte incompatibility may be present in many granulocyte transfusions.

14.12 Reactions due to bacterial contamination

Transfusion-transmitted bacterial infections

It has been known for years that a small percentage of units of whole blood contain viable bacteria. Transmission of bacterial infection was a major problem in the early days of blood transfusion [83–87]; however, improvements in blood containers, the development of the closed system for producing blood components, and storage at refrigerator temperatures were thought to have virtually eliminated this problem. With the storage of platelets for 7 days at room temperature, the problem of bacterial contamination and transfusion transmission infection resurfaced [88–92]. The storage time of platelets was reduced to 5 days and the issue of bacterial contamination seemed to disappear. However, during the past few years, concern about transfusion-transmitted bacterial infection have been renewed once again and deaths due to contaminated blood components reported to the FDA account for about 12% of transfusion-related fatalities [8, 93]. The scope of the problem is difficult to ascertain [94, 95]. The rate of contaminated platelets ranges from 8 to 80 per 100,000 whole-blood-derived platelets 0 to 230 per 100,000 for apheresis platelets, and 0 to 3 per 100,000 units of red cells [96–103]. The magnitude of this clinical problem is difficult to define because only the more severe reactions are reported [104–110] and other factors are important such as the patient's underlying condition and the number and type of bacteria or presence of endotoxin in the blood component. Transfusion-transmitted bacterial infection of clinical importance may occur about 1 per 25,000 units of platelets and 1 per 250,000 units of red cells [104–110]. The fatality rate is difficult to determine, but 5–8 fatalities

Table 14.7 Strategies to prevent transfusion-associated sepsis.

Avoid bacterial contamination
   Defer donors with dental procedures/gastroenteritis
   Good skin preparation at venipuncture site
   Single-use sterile needle and blood container
   Keep outside of blood container clean
   Phlebotomist hand washing
   Divert first 15–30 mL blood during collection
Component storage
   Red cells at 1–4°C
   Transfuse thawed frozen components within 4 hours
   Maintain clean water bath for thawing components
   Use sterile procedure for pooling components
Detection (under development)
   Visual inspection of components before issue
Direct bacterial culture (manual or automated)

are reported to the FDA annually [8]. Over half of the deaths are due to contaminated units of red cells, with platelet contamination being somewhat more common than plasma contamination. Of the contaminated red cells, half are caused by *Yersinia enterocolitica* [93].

There are two general types of concerns: bacterial contamination of platelet concentrates stored at room temperature and transmission of bacteria, especially *Y. enterocolitica*, from red cells stored at refrigerator temperatures [111]. For either of these situations, the sources of potential contamination include the donor's blood, the skin at the venipuncture site, and the environment such as air, equipment, water, and the phlebotomist and rarely the blood collection pack. Low-level bacteremia can occur following routine dental procedures and these individuals are temporarily deferred as blood donors [111]. The type of organism involved can provide a clue about the source of bacteria. For instance, *Staphylococcus epidermidis* is among the normal skin flora and suggests contamination from the venipuncture site, whereas Pseudomonas suggests water or equipment contamination. Steps taken to minimize the possibility of transfusion-associated sepsis include avoiding contaminating the unit during collection by effective skin decontamination and diversion of the first 20–30 mL of blood (see Chapter 4), proper storage and handling conditions, and detection of bacteria before transfusion (Table 14.7). Most patients can clear a rather large number of organisms; for instance, 10^4 coagulase-negative staphylococci.

The contamination of red cell units with cold-growing organisms is rare and usually involves *Y. enterocolitica* [111–116]. *Y. enterocolitica* can grow at temperatures below 37°C and in a calcium-free medium, thus making it more suitable for proliferation in refrigerated anticoagulated blood. There is a rather long lag phase of growth, and this is consistent with the observation that most units contaminated with *Y. enterocolitica* have been stored 20 days or longer [93, 111]. Although the organism loses some of its

virulence during growth, the endotoxins produced can have a severe clinical effect. *Y. enterocolitica* infection in normal, healthy individuals is often asymptomatic or may be associated with mild gastrointestinal symptoms. Questioning donors about gastrointestinal symptoms in the preceding 30 days is not an effective method of preventing the collection of blood from donors who might have circulating organisms because only about 50% of infected donors recall any symptoms and 11% of otherwise healthy suitable donors do report minor symptoms.

The signs and symptoms of septic transfusion reactions are variable but usually involve chills and fever beginning, during, or shortly after the transfusion. Other typical signs of sepsis may also occur such as hypotension, nausea, vomiting, oliguria, shock, respiratory symptoms, or bleeding due to DIC. Some contaminated units contain a small number of organisms and do not cause a clinical reaction [91], but more severe reactions involve fever, hypotension, or other symptoms of sepsis [93, 117, 118]. Thus, when evaluating a transfusion reaction it is important to consider that the blood component might be contaminated. The likelihood that a febrile reaction is due to contamination is greater if the temperature elevations are more than 2°C [93]. The unit should be cultured and if contamination seems likely on clinical grounds, antibiotics should be instituted while culture results are pending. Fatal reactions are more likely to be due to Gram-negative bacteria [117].

Several systems have been suggested to prevent transfusion of contaminated blood components (Table 14.7). Kim et al. [119] showed that red cells contaminated with *Y. enterocolitica* have a noticeably different color and can be identified visually. Yomtovian carried out a surveillance system using Gram staining [120]. These and measurement of pH or glucose have not been adopted because they are not very sensitive to detection of small numbers of bacteria. The method widely used at this writing is the culture systems that are used for patient blood cultures [121]. Drawbacks to this approach are that the sample is taken after several hours to provide time for bacteria to proliferate and reach a detectable level and then the culture requires about 18 hours. Thus, the testing usually involves a 1-day delay in product release. In addition, culturing individual units of whole-blood-derived platelets is expensive and this has led to further decline in their use. Alternative systems are under development to test products at the time of transfusion when the level of organisms is higher and thus more easily detected. Current bacterial detection systems do not detect all contaminated units and they have not eliminated transfusion-related septic fatalities [8, 122–124].

14.13 Signs, symptoms, and management of a transfusion reaction

Signs and symptoms of a transfusion reaction

The monitoring and nursing care that should be provided to patients receiving a transfusion are described in Chapter 13. It is not possible to

Table 14.8 Signs and symptoms of a hemolytic transfusion reaction.

Fever	47.5%
Fever and chills	40%
Chest pain	15%
Hypotension	15%
Nausea	5%
Flushing	5%
Dyspnea	5%
Hemoglobinuria	2.5%
Others	
Low back pain	NA
Abdominal pain	NA
Vomiting	NA
Diarrhea	NA
Unexpected bleeding	NA

Source: Pineda AA, Brzica SM, Taswell HF. Hemolytic transfusion reaction: recent experience in a large blood bank. Mayo Clin Proc 1978; 53:378.
NA, not available

predict the cause or ultimate severity of a transfusion reaction from the presenting signs and symptoms. Therefore, all patients who exhibit signs or symptoms during or within approximately 4 hours after transfusion should be managed initially as if a transfusion reaction were occurring. The most common signs and symptoms are chills, fever, and urticaria (Tables 14.5 and 14.8 and Figure 14.1). Because the signs and symptoms of different kinds of transfusion reactions vary widely, they usually do not accurately define the type of reaction that is occurring and so patient management focuses on the specific clinical problems present. Symptoms more likely to be associated with different kinds of transfusion reactions are presented in Table 14.9.

Initial steps in the management of a transfusion reaction

When a transfusion reaction is suspected, the steps listed in Table 14.10 should be taken. The transfusion should be stopped immediately to avoid transfusing additional blood. However, the needle or catheter should be left in the vein to maintain a route for administration of medications or fluids. If the patient becomes hypotensive, it may be difficult and time consuming to restart another intravenous route. Infusion of normal saline should be begun through the intravenous line. Vital signs should be obtained, including temperature, pulse, respiratory rate, and blood pressure. A brief physical examination should be carried out, including auscultation of the lungs and heart, inspection of the skin for hives, and inspection of the patient for signs of abnormal bleeding. A new blood sample should be obtained for repeat red blood cell compatibility testing and inspection of the plasma for evidence of hemolysis. A urine sample should be obtained if the patient can void, and a chest X-ray should be performed if pulmonary symptoms are prominent. The blood container and attached tubing and

Table 14.9 Symptoms related to different transfusion reactions.

Type of reaction	Associated symptoms
Acute hemolytic reaction	Restlessness
	Anxiety
	Severe chill
	Rapid temperature increase
	Headache
	Pleuritic pain
	Lumbar or thigh pain
	Hemoglobinuria
	Nausea
	Vomiting
	Pulse rate increase
	Blood pressure increase
	Oliguria
	Feeling of impending doom
Febrile (leukocyte) reaction	Chill
	Temperature increase greater than 1°C
	Nausea
	Muscle aching
Allergic reaction	Rash
	Hives
	Facial swelling
Respiratory distress	Sudden dyspnea
	Cardiac overload
	Cyanosis
	Cough
	Frothy sputum
	Blood pressure increase
	Distended neck veins
	Central venous pressure increase
Bacterial contamination	Fever
	Hypotension

filter should be returned to the blood bank and stored in the refrigerator until the cause of the reaction is established. Thus, if bacterial contamination is suspected, a sample can be removed in the laboratory from the bag and sent for culture. A Gram stain may detect a heavily contaminated unit but will also have many false negative results.

Initial treatment of a transfusion reaction

At this point it is possible to make a preliminary assessment of the situation to decide if more specific treatment is needed. If during the performance of these steps the patient's condition deteriorates, specific measures should be taken to deal with the problems. Respiratory problems may require epinephrine, nasal oxygen, and/or intubation. Hypotension should be treated with infusions of crystalloid. Red or pink plasma or serum suggesting hemolysis necessitates accurate monitoring of urine flow.

Table 14.10 Steps to take when a transfusion reaction occurs.

1. Stop the transfusion immediately
2. Leave the needle in the vein and begin infusing normal saline
3. Obtain vital signs of temperature, pulse, respiratory rate, and blood pressure
4. Begin oxygen administration if pulmonary symptoms are prominent
5. Carry out a brief physical examination; auscultate the lungs and heart; inspect the skin for hives; inspect the patient for signs of abnormal bleeding
6. Obtain a new blood sample for repeat red blood cell compatibility testing and inspection of the plasma for evidence of hemolysis
7. Obtain a urine sample if the patient can void
8. Obtain a chest X-ray if pulmonary symptoms are prominent
9. Make a preliminary assessment of the situation
10. Begin definitive treatment based on initial assessment

If necessary, the patient should be catheterized. If there is evidence of oliguria, mannitol should be added to maintain urine volume at approximately 100 mL/hour. If the patient demonstrates only hives, he or she can be given diphenhydramine 50 mg intramuscularly and the transfusion restarted slowly after about 15 minutes.

14.14 Immunologic complications of transfusion

Immunization to blood group antigens
As a result of exposure to blood, patients may form antibodies to red cell; lymphocyte, granulocyte, or platelet surface antigens; or plasma proteins. The likelihood of antibody formation depends on the immunogenicity of the antigen and the ability of the individual to mount an antibody response. Each kind of antibody can cause a particular clinical problem later if the patient requires subsequent transfusions or organ or tissue grafts, or becomes pregnant (Table 14.11).

Alloimmunization to red cells
The incidence of formation of RBC antibodies varies with the population studied; thus, no suitable overall figure is meaningful. General hospitalized patients, pregnant women, oncology patients, or patients who receive multiple transfusions such as sickle cell disease or thalassemia can be expected to have different prevalence of RBC antibodies. In an active follow-up study of patients undergoing elective surgery, Redman et al. [125] found newly formed red cell antibodies in 8.3% of patients. The patients received an average of three units of red cells, and the antibodies developed within 2–24 weeks. Many antibodies were detected initially only with enzyme-treated test red cells, but most became active in the antihuman globulin phase. Most (76%) of the antibodies had Rh specificity. The likelihood of developing an antibody was similar for males (8.2%) and females (8.6%) and is not related to the length of storage of the

Table 14.11 Kinds of clinical problems caused by immunization after blood transfusion.

Antibody target	Possible clinical problem
Red cells	Hemolytic reactions
	Difficulty locating compatible blood if transfusion needed
	Hemolytic disease of the newborn
Lymphocytes (HLA)	Febrile reaction
	Difficulty locating compatible organ if transplant needed
	Poor response to platelet transfusion
Granulocytes	Febrile reaction
	Acute pulmonary reaction
	Poor response to granulocyte transfusions
	Alloimmune neonatal neutropenia
Platelets	Poor response to platelet transfusions
	Alloimmune neonatal thrombocytopenia
	Posttransfusion purpura
Plasma proteins	Allergic reaction
	Anaphylactic reaction (IgA)

Source: Reproduced with permission from McCullough J. Transfusion medicine. In: Handin RI, Lux SE, Stossel TP, eds. Blood: Principles and Practice of Hematology, 2nd ed. Philadelphia: Lippincott, Williams and Wilkins, 2003, p. 2055.

transfused red cells. Although immature or newborn infants rarely make red cell alloantibodies, anti-D can be formed in D positive infants with exposure to the small amount of red cells (0.6 mL) in whole blood-derived platelets [126].

Alloimmunization affecting platelet transfusion

Alloimmunization to platelets or leukocytes that occurs during pregnancy or transfusion can cause a poor response (refractoriness) to platelet transfusion (see Chapter 11). This is a very major problem in platelet transfusion and can be considered a complication of transfusion. Platelet refractoriness occurs in about 20% of multitransfused patients [127]. The use of leukodepleted blood components [127–130] has greatly decreased this problem.

Alloimmunization following granulocyte transfusion

The rate of alloimmunization from granulocyte transfusions ranges from 12% to 88% [131]. This may be manifested as either HLA or granulocyte-specific antibodies. These antibodies interfere with intravascular survival and tissue localization of transfused granulocytes and thus are clinically very significant [132] (see Chapter 11).

Transfusion-associated graft-versus-host disease

Transfusion-associated GVHD is caused by viable alloreactive T-lymphocytes contained in the blood components [133–142]. This is discussed extensively in Chapters 11 and 12. In patients who are severely immunocompromised, transfused lymphocytes proliferate, causing a

Table 14.12 Clinical and pathologic comparison of graft-versus-host disease associated with bone marrow transplantation (BMT-GVHD) and transfusions (PT-GVHD).

Manifestation	BMT-GVHD	PT-GVHD
Time sequence	35–70 days	2–30 days
Skin rash	+	+
Constitutional symptoms	Profound	Mild to moderate
Liver enzyme elevation	+	+
Pancytopenia	Rare to minimal	Almost always
Bone marrow hypoplasia or aplasia	Negative	Positive
Occurrence of GVHD	70%	0.1–1.0%
Response to therapy	80–90%	None
Mortality	10–15%	90–100%

Source: Brubaker DB. Transfusion-associated graft-versus-host disease. Hum Pathol 1986; 17: 1085–1088.

syndrome characterized by fever, liver dysfunction, skin rash, diarrhea, and marrow hypoplasia. The syndrome begins less than 30 days following transfusions and is fatal in approximately 90% of patients [142]. The cellular mechanisms involved in transfusion-associated GVHD are assumed to be similar to those in GVHD due to allogeneic bone marrow although there are differences in the disease manifestations (Table 14.12). Recently GVHD has been reported in immunocompetent patients. It appears that they develop GVHD after receiving blood from a donor who is homozygous for one of the recipient's HLA haplotypes, and thus the donor's leukocytes are not recognized as foreign and are not destroyed [144–146]. Transfusion-associated GVHD can be prevented by irradiating the blood components prior to transfusion (see Chapter 11).

Transfusion-related immune modulation

In addition to providing antigens to stimulate alloimmune response, blood transfusion has other immunomodulating effects [147, 148]. These immunomodulatory effects may involve transplantation, cancer recurrence, susceptibility to infection, or virus activation [149] but also initiate proinflammatory mechanisms leading to multiorgan failure or increased mortality [148]. The clinical impact of transfusion-related immunomodulation (TRIM) is difficult to define. The effect is probably due to allogeneic leukocytes and thus might be abrogated by the use of leukocyte-reduced or autologous blood. However, understanding of TRIM and its clinical consequences are not sufficiently well known to warrant recommendations to alter blood component composition or transfusion therapy. More detail of some TRIM situations is as follows.

Alteration of graft survival

Blood transfusion has two opposite effects on allograft success depending on the particular allograft. In 1973, Opelz et al. [150] demonstrated that transfusion to patients awaiting a kidney transplant resulted in a better

outcome of transplantation than occurred in nontransfused patients. This enhancing effect of transfusion was also seen in living related transplants [150, 151]. Thus, an immunologically beneficial effect of transfusion was demonstrated. It is not clear whether the effect is observed today because of the improvements in kidney transplant management [151], but the biological principle has been established. Based on animal studies, the proposed mechanisms of the immunomodulatory effect of blood transfusion include clonal deletion, active suppression, and host anergy [152]. Blood transfusion can induce tolerance in a transplant setting, and this effect is not present if the leukocytes are removed.

In contrast, transfusion has a detrimental effect on the outcome of marrow transplantation. The presence of HLA antibodies is associated with marrow graft rejection [153], and pretransplant transfusions are associated with reduced patient and graft survival [154, 155]. Thus, for marrow transplantation the biological effect is the opposite of that seen with kidney grafts. The mechanism in marrow transplants seems to be a rather straightforward alloimmunization that causes rejection of the transplanted stem cells. However, the pathophysiological mechanism that provides the opposite effect for kidney grafts remains unknown. This demonstration of a reduced immune response may be relevant to the growing concerns that transfusion may render the recipient more susceptible to infection or recurrence of malignancy (see below).

Increased susceptibility to recurrence of malignancy

In mice, transfusion of allogeneic blood before infusion of tumor cells accelerates tumor growth and promotes metastasis [156]. This effect can be reduced by the transfusion of blood depleted of leukocytes [156], although the leukocytes must be removed from the blood before it is stored [157], suggesting that leukocyte products generated during storage can promote tumor growth and the effect is mediated by a cytokine [152].

Blajchman [147] points out that more than 150 studies have reported on the relationship (or lack thereof) between transfusion and cancer recurrence or postoperative infection. Despite this large amount of data, the results are not clear. Vamvakis [158] summarized 60 different clinical studies of the effect of transfusion on cancer recurrence and found that 28 of these studies concluded that transfusion was associated with an increased likelihood of cancer recurrence. Rather than cite a few of the studies here, the reader is referred to Vamvakis' [158] summary for all 60 references. The crude data summary showed a significantly increased relative risk of recurrence for all cancers studied except cervical cancer [148]. However, three other studies reached different conclusions. Busch et al. [159] found that the relative rate of recurrence of colorectal cancer was about two times higher in patients receiving transfusions than in nontransfused patients. There were no differences between patients who received allogeneic blood and those who received autologous blood. Heiss et al. [160] also reported a deleterious effect of transfusion in colorectal cancer, but Houbiers [161] did not find an effect. Thus, studies have shown

conflicting results, but there is no good way of developing a controlled, randomized clinical trial [147]. As stated by Blumberg and Heal [162], "Perhaps the strongest scientific evidence that the transfusion immunomodulation theory may be relevant to human cancer is that the majority of animal experimental studies demonstrate a deleterious effect of allogeneic transfusions on cancer growth or metastasis." If this effect of transfusion is real, the clinical impact will be substantial. Blumberg estimates that the number of excess cancer deaths annually in the United States could be about 16,000 per million units of blood transfused [162]. If this effect can be prevented by depleting the blood of leukocytes, this will be a simple way to prevent a major transfusion complication.

Increased susceptibility to infection

The posttransfusion immunomodulation effect of blood transfusion might also lead to an increased susceptibility to infection. Three studies [163–165] showed an adverse effect of transfusion on postoperative infection, while others did not [161, 166]. In a meta-analysis of patients undergoing surgery for colorectal cancer, Vamvakis [167] found a 14% increased risk of postoperative infection when he used the confounding factors used in other studies but no increased risk when he adjusted for additional variables. He suggested that the effects reported by others might be the result of incomplete consideration of the variables that confound the situation, although adverse effects of TRIM "were remarkably consistent across most studies" [148]. Carson et al. [168] retrospectively analyzed data on more than 9000 hip fracture patients and reported an increased risk of serious bacterial infection was associated with transfusion. Chang et al. [169] found that the transfusion was an independent risk factor for postoperative bacterial infection in colorectal surgery patients. In a separate study of colorectal cancer surgery patients, Vamvakas et al. [170] found no overall relationship between transfusion and postoperative infection, but when adjusted for confounding variables, there was a 14% increased risk of infection per unit of red cells received. Thus, as in the case of cancer recurrence, we are left with conflicting data from human studies, rather solid data from animal studies indicating that transfusion can cause an increase in postoperative infections, but little likelihood that a true controlled trial comparing transfusion with no transfusion can be done. It seems that the conclusions of Blumberg apply to infection as well as to cancer recurrence. If the effect of transfusion on postoperative infection is real, this will have a substantial clinical impact. Blumberg estimates that for an infection rate of 15%, half of which is due to transfusion, an average of three units of blood per patient, and a mortality rate of 0.5% for postoperative infections, there would be 125 deaths per million units of blood transfused [162]. As with the effect on cancer recurrence, if the transfusion effect on infection is true and can be prevented by depleting the blood of leukocytes, this will be a very major step in improving blood safety.

Overall mortality

Several randomized, controlled clinical trials show a deleterious TRIM effect in patients undergoing cardiac surgery [148].

Microchimerism

Following transfusion, donor leukocytes briefly proliferate in recipients and disappear by 7–10 days [171]. This has been called transfusion-associated microchimerism (TAMC). It is now clear that TAMC may last for as long as 60 years [171] with 9.8% of transfused subjects demonstrating TAMC compared to 0.7% of controls [171–173]. Receipt of leukodepleted red cells does not appear to reduce the likelihood of developing TAMC. While long-term TAMC might be involved in autoimmune disorders, this has never been established. The aggregate of studies suggests that hematopoietic engraftment may occur in up to 10% of trauma patients who receive transfusion [171]. One study of 163 combat veterans, 9.8% of whom had TAMC, did not demonstrate any increased risk of autoimmune disease, coronary artery disease, or cancer [171]. This TRIM effect is not well understood and future studies should be interesting.

14.15 Nonimmunologic complications of blood transfusion

Hypothermia

Red cells are stored in the refrigerator, and if the transfusion is begun very soon after the unit is removed from the refrigerator, the patient will receive blood that is only slightly above 4°C. Transfusion of large volumes of cold blood is associated with cardiac arrhythmias and increased mortality [174, 175]. Transfusion of cold blood does not cause a clinical problem if the transfusion is being administered over 1 hour or at a flow rate in an adult of about 5 mL per minute or less. This flow represents about 1% of the normal 5000 mL per minute cardiac output, and the transfused cold blood is effectively mixed with the patient's body-temperature blood. If the transfusion is given rapidly, such as in massive transfusion or during cardiac surgery where blood may be transfused in large volumes near the coronary circulation, it is advisable to warm the blood [176] (also see Chapter 13).

Citrate toxicity

Whole blood is collected into citrate anticoagulant and so one of the potential complications of transfusion is citrate toxicity. Citrate toxicity manifests itself as hypocalcemia with symptoms of muscle paresthesias, twitching, anxiety, and, in more severe situations, seizures and cardiac arrhythmia [177–180]. Citrate toxicity is discussed in considerable detail in relation to apheresis (Chapter 7) because of the continuous flow of citrate back to the donor during apheresis. Those studies have established that rates of citrate administration up to 1 mg/kg/minute are well tolerated

[177–180]. These rates are rarely encountered in the transfusion of red cell, plasma, or platelet concentrates. In the preparation of red cells, most of the plasma is removed and the cells are resuspended in additive preservative solutions. Thus, most of the citrate anticoagulant has been removed because it travels with the plasma and platelet concentrate. Thus, the dose of citrate administered is usually not sufficient to cause citrate toxicity. Citrate toxicity is a greater concern in the transfusion of neonates, wherein even though the volume of blood administered is small, it may amount to a massive transfusion in relation to the patient's blood volume. Thus, it is customary to administer supplemental calcium during exchange transfusion. Citrate toxicity is also a complication in therapeutic plasma exchange, which is discussed in Chapter 19.

Bleeding tendency

Since very little plasma remains when red cells are suspended in the additive preservative solution, red cell transfusion does not provide coagulation factor replacement. Transfusion of blood that does not contain coagulation factors theoretically might lead to a bleeding tendency due to depletion of coagulation factors. This occurs in massive transfusion [181] and usually involves a depletion of platelets [182, 183] rather than bleeding due to depletion of coagulation factors (see Chapter 12). There seems to be little relation between the degree of bleeding and the level of coagulation factors [182–184], and, thus, unfortunately routine coagulation tests may not be very helpful in elucidating the cause of the bleeding [182, 185]. In the past, many blood banks have predetermined schemes for replacement of coagulation factors or platelets after certain volumes of blood have been used [183, 186, 187]. More recent military experience with massive trauma and hemorrhage has led to early use of plasma and platelets to prevent the development of coagulopathy (see Chapter 12).

14.16 Electrolyte and acid–base imbalance

Electrolyte imbalance is not much of a problem in practice. Theoretical concerns are that the patient might develop elevated potassium or ammonia levels or acidosis because of the composition of the stored red cells (see Chapter 5). This complication would be most likely to occur during massive transfusion (see Chapter 12), but fluid replacement strategies and overall management techniques for these patients have almost eliminated this as a problem. Electrolyte imbalance is also a concern in the transfusion of very small patients such as neonates, in whom even small volumes of red cells constitute a massive transfusion (see Chapter 12). Modern techniques now make it possible to avoid this potential complication.

14.17 Circulatory overload

When whole blood was used, circulatory overload was not uncommon. With the conversion to red cells, the additional unnecessary plasma has

been eliminated from most transfusions, and this has almost eliminated circulatory overload. Certainly some patients who have very compromised cardiac function, or those with massive blood loss or fluid shifts in whom it is difficult to maintain a proper blood volume, may experience this complication.

14.18 Iron overload

Patients who receive chronic transfusion therapy may become overloaded with iron and develop iatrogenic hemochromatosis (see Chapter 12). Iron chelators, supertransfusion, and neocytes have been used, but this problem remains a serious potential complication.

14.19 Embolism

In the early days of transfusion therapy, emboli of rubber plugs (from the needles passing through the rubber stoppers of the glass bottles), pieces of intravenous tubing, and other particulate matter and air occurred. As the techniques of transfusion improved and modern equipment was developed, these complications have almost disappeared. Occasionally a piece of a catheter may dislodge, but this is really a complication of the vascular access device being used. Air embolism is still a theoretical complication of blood cell separators used for apheresis (see Chapters 7 and 19), but this is a donor complication and is not considered a complication of transfusion. During the 1970s and 1980s, concern developed that the microaggregates that form in stored red cells might act as microemboli, and at one time these were thought to be involved in adult respiratory distress syndrome (ARDS) [188–190]. Microaggregate filters were developed to prevent this problem. However, the relationship between microaggregates and ARDS was never clearly established, and microaggregate filters are not widely used today [188]. One reason for this is the trend to use leukodepleted components for patients in whom contaminating leukocytes are thought to pose a problem (see Chapter 11). This avoids the development of microaggregates if the leukocytes are removed soon after the blood is collected. In 2002, observation of large white particulate matter in units of red cells caused great concern until it was established that these were large aggregates of platelets and fibrin [191–195] that are removed by routine blood administration filters.

14.20 Passive transfer of hypersensitivity

Massive transfer of delayed hypersensitivity in the form of a positive skin test to tuberculin PPD can occur [152].

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15 Transfusion-Transmitted Diseases

The public and patients have great interest and concern about contracting a disease as a result of blood transfusion. This has been driven primarily by the public's fear of AIDS. The result has been a great change in the nature of transfusion practice, in the regulation of blood banks, and in the organization and operation of blood collection organizations [1, 2].

   Several strategies are discussed throughout this book that are used to reduce the risks of transfusion (Table 15.1). Major changes have been made in the selection of blood donors (Table 15.2). Eligibility criteria have been changed to reflect the understanding of behavior that places potential donors at risk of transmitting disease. The number of donor screening questions is extensive and the questions are quite specific about risk behaviors. Laboratory testing of donated blood has also undergone extensive change. For most of the first 50 years of blood banking, blood underwent one or two tests (first for syphilis; later, also for hepatitis B antigen). Following the introduction of the test for human immunodeficiency virus (HIV), several additional tests were implemented including tests for viral nucleic acid. These steps have been extremely effective in reducing the risks of blood transfusion (Table 15.3) [3–15]. This chapter discusses the risks of disease transmission by transfusion and the approaches being taken to minimize those risks. Transfusion of bacterially contaminated blood is discussed in Chapter 14.

15.1 Syphilis

Transmission of syphilis by blood transfusion was common in the early days of blood transfusion, but now it is extremely rare [16] with no cases of transfusion-transmitted syphilis reported in the last 35 years [17]. The treponeme survives in refrigerated blood for only 48–96 hours [18]. Thus, theoretically syphilis can be transmitted from refrigerated components stored only a few days or from platelet concentrates stored at room temperature, but studies of contemporary blood component storage conditions have not been done. Syphilis is a major worldwide infection and is still prevalent in the United States. Syphilis confirmed positive donations range from 0.011% to 0.13% [19–22]. All blood donors are tested for syphilis; however, this is not a very effective method of preventing transfusion-transmitted syphilis in the United States [16], and *Treponema*

Table 15.1 General strategies to reduce disease transmission.

Improved donor selection
Improved transmissible disease testing
Reduced donor exposure
Use of autologous blood
Use of directed donors
Limited-donor programs
Decreased blood use
Changed indications for transfusion
Pharmacologic stimulation or substitution
Modified the blood component
Inactivation of viruses and bacteria

pallidum cannot be detected in the blood of donors with a positive screening test using nucleic acid amplification techniques [17]. Serologic tests for syphilis are negative 50% or more at the time of spirochetemia in persons with primary syphilis. Only half of donors with a positive screening test report a history of syphilis [23]. In the mid-1980s, it was recommended that syphilis testing be abandoned, but the test was retained as a surrogate marker for sexually transmitted diseases because of the possibility that this would identify donors at risk for transmitting HIV. This did not prove to be so [22], but in the absence of laboratory data establishing that fresh blood components or those stored at room temperature are not infectious, the test requirement was retained. With the present general concern regarding the safety of the blood supply, it is unlikely that the requirement for routine syphilis testing will be eliminated in the absence of good laboratory data to support the decision [24, 25].

15.2 Hepatitis

Posttransfusion hepatitis is the most common disease transmitted by blood transfusion. Posttransfusion hepatitis can be caused by hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), or Epstein–Barr virus (EBV) or may be defined as non-A, non-B, non-C, which means hepatitis due to none of the agents listed above. Estimates of the frequency of posttransfusion hepatitis are complicated because this depends on the blood donor population and when the studies

Table 15.2 Blood bank procedures to reduce the infectivity of the blood supply.

Recruitment health criteria
Medical history
Physical examination
Donor deferral registry
Laboratory tests
Donor callback

Table 15.3 Estimates of transfusion-transmitted disease in the United States. One case of each disease would be expected following the number of units of blood shown.

	Lackritz 1995	Sloand 1995	Williams 1997	Dodd 2002	Zou 2009	Tabor 2002	O'Brien 2007	Stramer	Zou	Total US Cases[a]
Hepatitis C	–	3,300	103,000	1,935,000	–	625,000	2,300,000	230,000		6
Hepatitis B	–	50,000	63,000	–	282,000–355,00	150,000	153,000	–		48[b]
HTLV-I/II	–	69,272	641,000	–	–	–	–	–		20
HIV	450,000–660,000	40,000–400,000	493,000	2,135,000	–	769,230	7,800,000	3,100,000		5

Source: References [3–13].

[a]Calculations based on transfusion of 12,000,000 units of blood annually and Dodd incidence figures.
[b]Calculations based on data from Strong and Katz.

were done. There was a substantial reduction in the incidence of posttransfusion hepatitis in the United States in the early 1970s because of the conversion from paid to volunteer donors [26]. Posttransfusion hepatitis remains a major health problem. Based on the estimated number of persons transfused in the United States annually and the proportion of those who survive the illness that necessitated the transfusion, it is estimated that 120,000 cases of posttransfusion hepatitis occurred annually during the 1980s [26].

Hepatitis A

Hepatitis A usually has a short period of viremia that occurs for about 7 days before the onset of acute symptoms. Although about 10% of patients may have a relapsing course for up to 1 year, hepatitis A generally does not involve a carrier state, and thus there is no chronic viremia. Although posttransfusion hepatitis A is rare, it can occur if a donor is unaware of the hepatitis A exposure and donates blood during the few days of viremia before symptoms develop [27, 28]. However, because of the absence of hepatitis A antibody at the time of viremia, the lack of a practical test for the HAV itself, and the rarity of posttransfusion hepatitis A [29, 30], laboratory testing of blood donors for hepatitis A is not done. Thus, in evaluating a patient with suspected posttransfusion hepatitis, hepatitis A should be considered but as a very remote possibility.

Hepatitis B

The discovery of the HBV from studies in Australia [31] provided the first major step in reducing transfusion-transmitted infections. The biology of HBV infection makes it a dangerous transfusion-transmissible disease [32]. The most common response to infection with the HBV is asymptomatic (Figure 15.1). If the individual does develop symptoms, viremia and HBsAg positivity occur 2–6 weeks before the onset of symptoms; thus, an infectious but apparently healthy individual may meet all of the donor medical history and laboratory testing criteria and donate
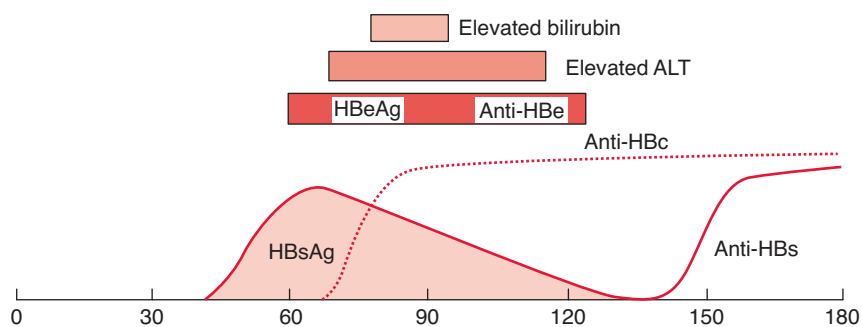
Figure 15.1 Serologic and biochemical events in acute hepatitis B. (Reproduced with permission from Dodd RY. Hepatitis. In: Petz LD, Swisher SN, Kleiman S, et al. (eds). Clinical Practice of Transfusion Medicine, 3rd ed. Oxford: Churchill Livingstone, 1996, pp. 847–873 (Fig. 38-1).)
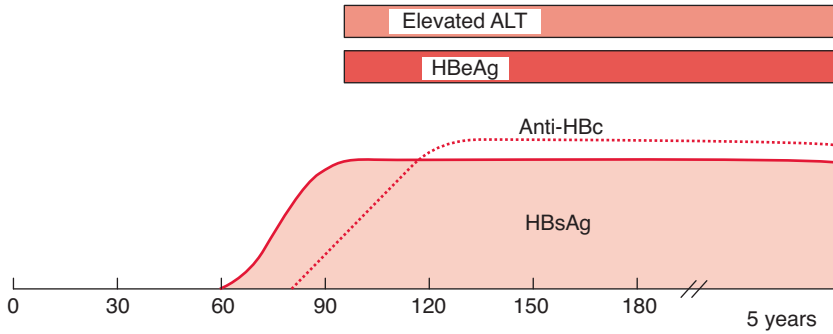
Figure 15.2 Serologic and biochemical events in chronic hepatitis B/HBsAG carrier state. (Reproduced with permission from Dodd RY. Hepatitis. In: Petz LD, Swisher SN, Kleinman S, et al. (eds). Clinical Practice of Transfusion Medicine, 3rd ed. Oxford: Churchill Livingstone, 1996, pp. 847–873 (Fig. 38-2).)

a unit of infectious blood. Individuals who develop symptomatic HBV infection would be screened out from subsequent blood donations on the basis of their medical history. About 90% of acute HBV infections clinically resolve, the virus disappears, and the individual becomes HBsAg negative (Figure 15.1). Antibody to the hepatitis B virus (anti-HBs) develops in almost all (80–90%) of these individuals. However, in about 10% of patients, chronic HBV infection develops, the virus persists, and the individual remains HBsAg positive (Figure 15.2). If any of these individuals have experienced a subclinical (asymptomatic) initial infection, they would pass the medical history, but their blood should be found to be infectious by the HBsAg testing done prior to the release of blood for transfusion.

In the past, hepatitis B was an major complication of blood transfusion [26, 33]. The adoption of routine screening of blood donors for hepatitis B surface antigen reduced the incidence of posttransfusion hepatitis B, and now it is estimated to occur about once for every 250,000–350,000 units of blood or about 48 cases per year (Table 15.3). However, this is still the most common form of posttransfusion hepatitis. In 1987, routine screening of blood donors' antibody to the hepatitis B core antigen (anti-HBc) was introduced in an effort to reduce the transmission of non-A, non-B hepatitis. However, an additional benefit of this screening was the further reduction of transfusion-transmitted hepatitis B, since HBsAg negative anti-HBc positive blood can transmit HBV [34–37].

The implementation of nucleic acid amplification testing (NAT) for HCV and HIV (see later) has reduced the transfusion risk from these viruses so greatly that concern is focused increasingly on HBV [37]. NAT has not been implemented for HBV. HBsAg negative donors have very low levels of circulating viruses that are not detectable in NAT [36–38].

Non-A, non-B hepatitis and surrogate testing
In the 1970s and 1980s, non-A, non-B hepatitis was a diagnosis of exclusion, referring to hepatitis not caused by HAV or HBV. There was

considerable debate as to whether non-A, non-B hepatitis had a major long-term health impact, but this was resolved with the observation that in patients with posttransfusion hepatitis, 50–70% had liver function abnormalities for 1–3 years [38]. Of those who underwent liver biopsy, 90% had either chronic active or chronic persistent hepatitis or cirrhosis. In 1986, blood banks in the United States instituted routine testing of donors for alanine aminotransferase (ALT) and hepatitis B core antibody (HBc) as a way of reducing posttransfusion non-A, non-B hepatitis. The use of these tests—which are not specific for non-A, non-B hepatitis—was controversial. In 1981, data from the transfusion-transmitted virus study showed that there was an association between elevated ALT and posttransfusion non-A, non-B hepatitis [33] and that excluding blood with an elevated ALT level would reduce posttransfusion non-A, non-B hepatitis by 30%. A similar study at the National Institutes of Health gave a similar result [39]. However, neither study was randomized and blood with elevated ALT was not excluded from use; thus, the effect on reduction in posttransfusion non-A, non-B hepatitis was a calculated projection, not an actual observation. Later, the transfusion-transmitted virus study data were analyzed to determine whether there was also an association between anti-HBc and posttransfusion non-A, non-B hepatitis [34]. This and National Institutes of Health data [39] showed that posttransfusion non-A, non-B hepatitis was more than twice as frequent in recipients of anti-HBc-positive blood compared with anti-HBc-negative blood. It was projected that eliminating blood that was anti-HBc positive would eliminate about 40% of posttransfusion hepatitis. The ALT and anti-HBc tests have very little overlap in the individuals who are positive and so seem to identify two different groups of potentially infectious donors.

In Canada, surrogate testing was not implemented and this provided an opportunity to evaluate the testing the effect of ALT and anti-HBc testing of donated blood in reducing posttransfusion hepatitis [40]. From 1988 to 1992, 4588 patients who received allogeneic blood were enrolled in a study in which they received blood from which units that tested positive for either surrogate marker (ALT or anti-HBc) were either withheld or not withheld. Withholding blood that tested positive for one or both markers decreased posttransfusion hepatitis by 40% [40]. Since this study took place during the time that HCV testing was implemented, it was possible to determine the effect of surrogate testing on hepatitis C as well as on non-A, non-B, non-C hepatitis. Most of the benefit of surrogate testing was in the reduction of hepatitis C before testing for anti-HCV was introduced. After the introduction of anti-HCV testing, the difference in posttransfusion hepatitis between the two groups of patients was not significant [40]. The availability of the anti-HCV test has eliminated the effectiveness of the ALT testing. A consensus conference concluded that ALT testing is not useful in reducing posttransfusion hepatitis; another study confirmed this [22, 41], and its use has been discontinued. Anti-HBc testing of donated blood is continuing; however, it detects a few donors who are infectious for hepatitis B but have a negative HBsAg test [22, 37].

Hepatitis C

In 1989, an RNA virus similar in classification to a togavirus [42] was identified and termed hepatitis C because it accounted for most posttransfusion non-A, non-B hepatitis [43]. The identification of the virus has made it possible to determine the long-range effect of this disease. Acute hepatitis C is usually mild, with up to 80% of patients being asymptomatic [44]. However, the long-term effects are more serious because the virus tends to be persistent and develop into chronic liver disease [44–46].

A donor screening test for anti-HCV was introduced in 1992. Most donors who are anti-HCV positive have chronic hepatitis C regardless of their ALT levels [41, 47, 48]. Initially, anti-HCV-positive donors were more likely to be male, to be older, to have less than a high-school education, to be of black race or Hispanic ethnicity, to be a first-time blood donor, and to have been the recipient of a transfusion [49].

The impact of testing donors for anti-HCV was enormous [50]. It is estimated that the first-generation test prevented about 40,000 cases of posttransfusion hepatitis per year in the United States, and newer versions of the test, an additional 10,000–13,000 [44]. NAT has further reduced the risks of transfusion-transmitted hepatitis C. NAT was developed more quickly for HCV than other viruses because of the residual prevalence of the disease and the high level of circulating virus [51]. The risk of transfusion-transmitted HCV is now estimated to be only about half a dozen cases annually (Table 15.3). Some HCV seronegative donors are HCV positive by NAT only [52] and a few donors have very low levels of circulating virus [38, 53–55] and HCV can be transmitted by NAT-negative donors [56, 57]. Thus, serologic testing for HCV has continued.

Other hepatitis-related viruses

With discovery of the hepatitis A, B, and C viruses, it became clear that about 10% of transfusion-transmitted cases remained unclassified [58, 59]. A brief discussion of other viruses reported to be related to transfusion follows.

Hepatitis G

An RNA virus in the plasma of some who test negative for HAA, HBV, and HBC has been termed hepatitis G. There is a rather high rate of hepatitis G carriers (detected using polymerase chain reaction techniques) in the normal donor population, with rates ranging from 1% to 4% [58]. Hepatitis G RNA is found in some but not all patients with posttransfusion hepatitis who test negative for other hepatitis viruses, and it is clear that the virus is transmitted by transfusion [59, 60]. Since hepatitis G does not seem to complicate coexisting hepatitis B or C, or cause fulminant hepatitis [61], or make liver disease worse, there is some question about the role of this virus in liver disease. It has even been suggested that the designation "hepatitis" virus was premature, and definition of the biological effect of this virus must await further molecular and epidemiologic studies. At

present, there is no plan to screen blood donors for the virus. There is no practical test and no established role in disease transmission.

Hepatitis E virus

Hepatitis E virus (HEV) is endemic in some parts of the world and it can apparently be transmitted by transfusion [62]. However, posttransfusion HEV does not occur in the United States and its incidence in other parts of the world is not known.

TT virus

TT virus (TTV) was also originally thought to cause non-A-E hepatitis, but this virus is very prevalent in many countries and is not associated with hepatitis [63]. The TT was the original designation of the virus and does not stand for "transfusion-transmitted."

SEN virus

This is another virus that was proposed to be a cause of remaining non-A-E transfusion-transmitted hepatitis [64, 65], but it does not appear to play a role in hepatitis [64].

15.3 HIV infection and AIDS

First epidemiologic evidence [66], then clearer and extensive clinical [67] and laboratory evidence [68, 69] established that AIDS is caused by the HIV-1 retrovirus and can be transmitted by blood transfusion [68–72]. With the identification of HIV as the causative agent, it became clear that worldwide by the mid-1990s, millions of people had been infected with the virus, although a very small proportion of these individuals were infected through blood transfusion. With the introduction of screening of blood for HIV, transfusion transmission of the disease has been almost eliminated. Although there has been great concern about transfusion-transmitted HIV, transfusion accounted for less than 2% of all AIDS cases in the United States [73]. Only about 35 cases of transfusion-transmitted HIV have been identified after the implementation of screening in 1985 [73].

Because HIV was almost entirely limited to active male homosexuals, injecting drug users, or hemophiliacs exposed to clotting factor concentrate, it was possible to substantially reduce the infectivity of the blood supply through donor education and selective questioning to defer members of these risk groups. In 1983, blood banks altered their medical screening practices to defer potential donors from AIDS risk groups such as sexually active homosexual males and Haitian immigrants because of high prevalence of disease there. Another AIDS high-risk group, intravenous drug abusers, was already not acceptable donors. These steps were extremely effective in reducing the infectivity of the blood supply. For instance, in the San Francisco Bay area, the changes in donor eligibility criteria reduced the infectivity of blood by about 90% before the introduction of the laboratory test for the HIV virus (Figure 15.3) [74].
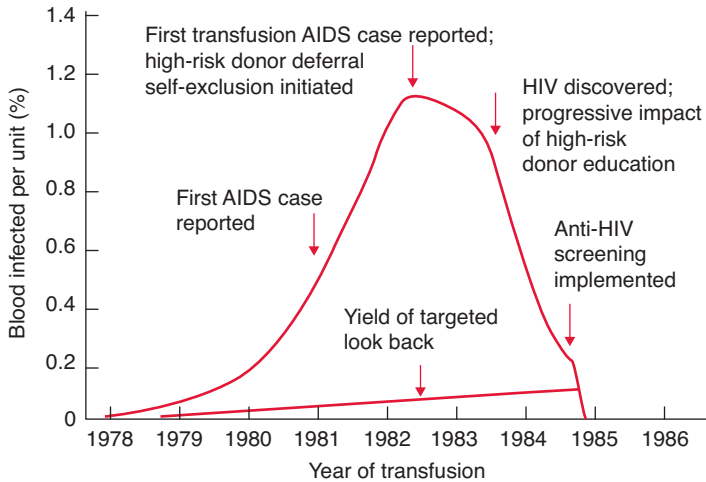
Figure 15.3 Impact of different donor screening procedures on the estimated infectivity of blood by the human immunodeficiency virus (HIV) in San Francisco, CA. Yield of targeted look back refers to detection of HIV cases by identifying recipients of previous blood donations from donors newly found to test positive for HIV. AIDS indicates acquired immunodeficiency syndrome. (Adapted with permission from Busch MP, Young MJ, Samson SJ, et al. Risk of human immunodeficiency virus (HIV) transmission by blood transfusions before the implementation of HIV-1 antibody screening. Transfusion 1991; 31:4–11.)

HIV laboratory screening tests

HIV-1 is a retrovirus and the proviral DNA is integrated into the host DNA, thus conferring permanent infection. Development of antibodies does not result in eradication of the virus but does signify previous infection and probable present infectivity. Although rare exceptions have been reported [75, 76], once present, anti-HIV persists until the individual becomes symptomatic. Thus, the test for HIV-1 antibody is used to detect infectious donated blood (Chapters 4 and 8).

HIV-1 antibody tests

The HIV-1 antibody test is an excellent test using an enzyme-linked assay. Although the specificity and sensitivity of the HIV antibody test is excellent, the predictive value of a positive HIV antibody test in blood donors is low because of the low prevalence of HIV infection in that population.

Risk of acquiring HIV by transfusion of anti-HIV-negative blood (window phase)

The interval between infection and the development of antibody to the infecting virus is known as the "window phase." Because the test for HIV detects anti-HIV, there is a window phase during which the individual is infectious but does not have a positive screening test for anti-HIV. Thus, despite testing for anti-HIV, transmission of the virus could still occur from blood donated during the window phase. It appears that the window

phase during which HIV-1 infection can be transmitted between infection and the appearance of antibody is about 6 weeks or 45 days [77–79].

HIV antigen testing

Since the virus should be present during the window phase prior to the development of antibody and since HIV antigen has been detected in some patients prior to their becoming HIV antibody positive, HIV antigen screening of blood donors was implemented to further reduce transmission of HIV by blood donation during the window phase. However, two large studies involving approximately 500,000 donors each did not identify any donors whose serum contained HIV antigen but no HIV antibody [80, 81]. On the basis of these studies, it appeared that HIV antigen testing would not be helpful and it has not been adopted.

Nucleic acid amplification testing

A different approach to reducing the window phase involves methods to amplify DNA or RNA sequences, thus making it possible to detect minute amounts of proviral DNA before the appearance of anti-HIV. At the urging of the FDA [82], NAT was developed and its implementation has led to the current very low incidence of transfusion transmitted HIV [83, 84] (Table 15.3). For a more extensive discussion of NAT, see Chapter 8.

Unfortunately, even NAT does not detect all infectious units of blood [85–87] and so a very small number of cases of transfusion-transmitted HIV still probably occur. In a summary of the first 3 years' experience with NAT of blood donors, positive tests for HIV were found in 0.27 per million donors [88]. With the introduction of NAT, the window period is 5–9 days or 1 in 2,300,000 donations [89]. Thus, the present risks of transfusion-transmitted infection would be about 1 in 2 million units of blood. This implies that about 6 infectious units may still enter the United States blood supply based on annual blood donations of 12–13 million. The introduction of NAT may have prevented 5 cases of transfusion-transmitted HIV and 56 cases of HCV [84] at a cost of about $2 million per infection prevented [90, 91].

Risk of acquiring HIV infection from transfusion of Anti-HIV-1-positive blood

The risk of acquiring HIV infection following transfusion with anti-HIV-1-positive blood is as high as 70–91% [92, 93]. Different components from an infected donor may have a different likelihood of transmitting HIV [94]. It has been estimated that prior to the introduction of HIV-1 antibody testing in May 1985, approximately 12,000 patients were infected with HIV-1 by transfusion [95, 96]. The incubation period between transfusion transmitted HIV-1 infection and the development of clinical AIDS is difficult to determine, but it may be from 4.5 to 14.2 years [97].

Effect of transfusion on AIDS

Blood transfusion appeared to be associated with a short survival in patients with AIDS [98] raising the question of whether this was cause or effect. A large-scale clinical trial, the Viral Activation Transfusion Study, showed that transfusion does not exacerbate other concomitant viral infections [99] or the HIV infection and has no overall detrimental effect to HIV infected patients [100].

15.4 Other transfusion-transmitted viruses

Although HIV and hepatitis are the agents of most concern, several other viral infectious diseases can be transmitted by transfusion (Table 15.4).

Cytomegalovirus

CMV is a herpesvirus that is common in the general population. In healthy individuals infection with CMV usually causes few or no symptoms. CMV can be transmitted by blood transfusion to both immunocompetent and immunodeficient patients (see Chapters 11 and 12 for extensive discussion). The earliest indications that CMV might be transmitted by blood transfusion were the observation of a mononucleosis-like syndrome that occurred several weeks after open-heart surgery in immunocompetent patients. This became known as postperfusion or postpump syndrome. The heterophil-negative cases were shown to be caused by CMV [101] and the heterophil-positive cases by EBV [101, 102]. Most of the immunocompetent patients who acquired transfusion-transmitted CMV

Table 15.4 Other transfusion-transmitted diseases of potential concern.

Agent	Disease	
Viruses	Parvovirus	RBC aplasia
Epstein–Barr virus	Mononucleosis	
Lymph malignancy		
HHV-6	Roseola; other?	
Cytomegalovirus[a]	CMV disease	
West Nile Virus	Febrile; meningoencephalitis	
HHV-8	Febrile; ? Kaposi sarcoma	
Chikengunya	Febrile; arthalgia	
Yersinia	Sepsis	
XMRV	? Chronic fatigue syndrome	
Parasite	*Plasmodium malariae*	Malaria
T. cruzi	Chagas' disease	
Tick Borne	Babesia	Babesiosis
Borrelia	Lyme disease	
Leishmania	Leishmaniasis	
Prions	Prions	Variant Creutzfeldt–Jakob disease

[a]For immunodeficient patients only.
? means "possible".

were asymptomatic; in contrast, CMV can be a serious viral pathogen in patients with either congenital or acquired immunodeficiency. Thus, posttransfusion CMV infection can range from asymptomatic with development of CMV antibody (in patients who are immunocompetent) to an infectious mononucleosis-like syndrome to severe, even fatal, generalized CMV infection [103–107]. Healthy individuals who pass all the medical screening requirements for blood donation may harbor CMV and transmit the virus via their donated blood components to susceptible patients. The CMV remains latent in the leukocytes of many infected healthy individuals, but efforts to isolate the virus have been unsuccessful 108, 109]. Thus, there is no practical laboratory test to determine which patients previously infected with CMV but presently healthy enough to donate blood may transmit the virus. The incidence of CMV antibodies in blood donors ranges from 30% to 80% and the presence of antibodies is used as a screening test. However, of these individuals only 2–12% have blood that may be capable of transmitting the virus [110]. Since the virus resides in the leukocytes, blood components depleted of leukocytes may have a reduced or even absent risk of transmitting CMV infection [111–115] (see Chapters 5, 11, and 13). Frozen deglycerolized red cells contain only about 1–5% of the original leukocytes and do not appear to transmit CMV infection [116], although washed red cells may transmit CMV [117].

Because it has been established that leukocyte depletion is effective in preventing CMV transmission, this is becoming the most common approach [118], although some CMV transmission occurs from leukodepleted blood [119]. Virus can be detected in plasma and this may account for the small residual risk of leukodepleted components [120]. The effectiveness of preventing transfusion-transmitted CMV in the specific situations in which CMV infection is a clinical problem is described in Chapters 11 and 12.

Human T-lymphotrophic virus I and II

Adult T-cell leukemia (ATL) was first recognized in Japan in the mid-1970s. Later the disease was shown to be caused by the human T-lymphotrophic virus (HTLV), which became the first retrovirus shown to cause malignancy in humans [121]. In ATL, there is peripheral lymphadenopathy, hepatosplenomegaly, skin lesions, abdominal pain, diarrhea, and abnormal pulmonary findings on chest X-ray. ATL disease is not very amenable to therapy. The HTLV-II virus is associated with a form of myelopathy and is referred to as HTLV-associated myelopathy or tropical spastic paraparesis (TSP) [121].

The virus and the ATL disease are endemic in Japan, the Caribbean Islands, parts of Central Asia, and Melanesia and also among the Australian aborigines. The virus can be transmitted vertically to newborns by maternal breast milk and also can be spread by parenteral and sexual routes [122–125]. Approximately two-thirds of initially reactive donor blood samples fail to react on subsequent testing, and only about 10% of the repeatedly reactive samples can later be shown to be infectious

[125, 126]. Of those donors with a true positive test for HTLV-I/II who are carriers, there is approximately a 1–5% chance of developing ATL during a 70-year life span if early deaths from other diseases are not considered. Another 2% may develop TSP 5–10 years later [127].

No cases of transfusion-transmitted ATL or TSP have been identified in the United States. However, transmission of the virus by blood transfusion does occur [128]. Approximately 60% of seronegative recipients developed anti-HTLV-I after transfusion of cellular blood products containing anti-HTLV-I [122, 124, 128]. In the United States, anti-HTLV-I is found almost exclusively in intravenous drug abusers or in persons from areas endemic for HTLV-I. The incidence of anti-HTLV-I in blood donors is 0.025% [129]. Because of the potential for disease transmission, routine testing of all donated blood for anti-HTLV-I was initiated in the United States in December 1988. This has been effective in avoiding the potential problem of transfusion-transmitted ATL or TSP.

Parvovirus

The parvovirus B19, implicated as a cause of aplastic anemia, has been transmitted by blood transfusion [130, 131]. Parvovirus is a common infection in children. Infected individuals may be asymptomatic or have a mild febrile illness. Infections apparently are infrequent in adults. The prevalence of parvovirus in blood donors is estimated to be between 1 in 3300 and 1 in 50,000 [132–134]. This, combined with the brief period of viremia, makes transmission of parvovirus by blood transfusion rare. However, because large numbers of units of plasma are used to produce derivatives, it is possible that parvovirus could contaminate derivatives, and since current viral inactivation processes are not effective against nonlipid enveloped viruses such as parvovirus, the infection could be transmitted. No parvovirus DNA was detected in 17 batches of albumin [132]; however, it appears that parvovirus can be transmitted by coagulation factor concentrates [134–136] and by solvent detergent plasma [137].

Epstein–Barr virus

EBV is the cause of heterophil-positive mononucleosis. Following infection with EBV, there is lifelong carrier state, and most adults have been infected. EBV can be transmitted by transfusion [138] and is one of the causes of the "postperfusion" syndrome—a viral-like illness occurring after transfusion of fresh blood during open-heart surgery [139, 140].
Transfusion-transmitted EBV has not been a major clinical problem, although it can be transmitted to organ recipients [138]. No donor screening or laboratory testing is done for EBV.

West Nile virus

West Nile virus (WNV) is a single-stranded RNA virus of the Flaviviridae family, first found in the West Nile area of Uganda. It remained in the Eastern Hemisphere (although widely distributed) until 1999 when it appeared in the United States [141, 142]. Following inoculation, most individuals have viremia for about a week and most infections are

asymptomatic. WNV fever is a typical viral illness with symptoms such as fever, headache, myalgia, fatigue, arthralgia, muscle weakness, nausea, or anorexia. As many as 10% of symptomatic patients may die usually of encephalitis. WNV is maintained in birds and mosquitoes, and the disease is transmitted to humans by mosquito bites. When WNV was found in the United States beginning in 1999, the biology of the virus suggested that it could be transmitted by transfusion. This was soon found to occur from transplanted organs [143], or blood transfusions [144–147]. Testing for IgM antibodies was begun, although this is not a satisfactory test for blood donor screening due to false-positive and false-negative results. A nucleic acid amplification test was developed with unprecedented speed and testing of blood donated from epidemic areas began in early summer 2003 [148–151]. While a few infectious units may not be detected due to low levels of viremia [152], NAT testing has been quite effective in reducing the likelihood of transfusion-transmitted WNV infection [148–151].

Toscana virus
This virus, found in the Mediterranean, causes meningitis and encephalitis similar to WNV. Thus, there are concerns regarding transmission by transfusion [153], but no steps involving the blood supply are being developed.

Human herpesvirus 6 and 8
Human herpesvirus 6 (HHV-6) causes a childhood febrile illness with a rash and is associated with a mononucleosis-like syndrome, certain autoimmune diseases, and lymphatic malignancies, and is an opportunistic infection in immunocompromised patients [154, 155]. HHV-6 is not presently considered a transfusion-transmitted disease. HHV-8 has been implicated in Kaposi sarcoma and other lymphatic malignances. There are very suggestive, but not definitive, data that HHV-8 can be transmitted by transfusion [156, 157]. No satisfactory interventions are available and a policy of "continuing active surveillance and data development" has been recommended [158].

Chikungunya virus
This mosquito-carried virus causes a febrile illness with particularly intense joint pain. Many cases are mild or asymptomatic, but severe, even fatal, infection can occur. The virus has been endemic in Africa, but in 2006 an outbreak occurred in islands in the Indian Ocean with cases spread to France and Southeast Asia. Although transfusion transmission has not been proven, it is theoretically possible. This led to the emergency establishment of pathogen inactivation of platelets [159]. A more recent outbreak in Italy [160] suggests that Chikengunya virus may become a more widespread and important transfusion-transmitted infection.

Simian foamy virus
This retrovirus causes persistent infection in nonhuman primates and while humans are not a natural host, simian foamy virus (SFV) antibodies

have been found in individuals who work with primates in zoos or research facilities. SFV does not seem to cause disease in humans, but parenteral transmission has been demonstrated in primates [161]. Considerable primate-human contact occurs in many countries either as part of the culture or from tourism. Thus, SFV could enter the United States blood supply and probably is already in the supply of some countries with primate-human contact. No intervention steps are being developed at this time.

Dengue virus

Dengue viruses are extremely widespread being the most common vector borne viral disease and endemic in about 40% of the earth. They cause a febrile illness ranging from asymptomatic to severe hemorrhagic fever and death. Because the viruses are transmitted by mosquito and many infected individuals are asymptomatic, transfusion transmission has occurred [162] and infected individuals are found increasingly in the United States not just in returning travelers but also from United States residents [163]. For instance, 28 cases of Dengue have been reported in Key West [163]. Thus, dengue is a serious potential threat to the United States' blood supply.

15.5 Transfusion-transmitted bacterial infections

This topic is discussed in Chapter 14.

15.6 Transfusion-transmitted parasitic and tick-borne diseases

Transfusion transmission of some parasites such as malaria has been known for years, and donor selection procedures are designed to identify potentially infectious donors. However, during the past few years a few cases of transfusion transmission of other parasitic and tick-borne diseases have been reported. As these diseases become more widespread [164, 165], there is a growing need to develop strategies to deal with this situation. There are no easy solutions. Many of the diseases have local or regional areas of prevalence, but as people move it is not be practical to limit donor selection procedures to those areas. In addition, there may not be suitable tests available for large-scale donor screening. Thus, approaches to dealing with these diseases will probably continue to evolve.

Malaria

From 1966 through 1999, 93 cases were reported to the Centers for Disease Control and Prevention, 11% of which were fatal [166]. All four species of Plasmodium can survive in refrigerated blood and have caused transfusion transmitted malaria, although most more recent cases involve *Plasmodium falciparum* [166]. Malaria has been transmitted by platelet transfusion as well as by red cells, and malarial parasites have been found in platelets of infected individuals. Thus, periodic spiking fever in a patient who has recently received a transfusion could be due to malaria, but this would be very unlikely.

Donors who might transmit malaria are screened out by medical history. *P. falciparum* and *Plasmodium vivax* produce symptoms within 6 months of infection. *P. falciparum* rarely persists longer than 1 or 2 years and *P. vivax* and *Plasmodium ovale* may persist rarely longer than 3 years. However, malaria has been transmitted by donors whose malaria exposure was 7 years (P. ovali), 13 years (*P. falciparum*), and 27 years (*P. vivax*) previous [166], but most individuals exhibit symptoms within 3 years of becoming infected. Thus, blood banks defer travelers returning from areas where malaria is endemic for 3 years. Unfortunately more than half of the cases reviewed by Mungai et al. [166] should have been but were not deferred by existing donor criteria. On the other hand, about 100,000 donors are deferred unnecessarily to prevent one case of transmitted malaria [167]. Laboratory testing of donors for malaria is not practical or cost-effective in the United States [168]. Examination of blood smear is done in many parts of the world as it is the only practical test available. However, this will not detect many infectious donors because they have a low level of parasitemia. Antigen detection tests are less sensitive and, although antibody testing falsely excludes many donors [166], this may be helpful in areas where malaria is prevalent [169].

Chagas' Disease

Trypanosoma cruzi, a parasite, is endemic in many parts of Central and South America, but only a few cases of infection in the United States have been reported. The organism causes Chagas' disease, in which patients may develop megacolon, megaesophagus, and heart failure. Once an individual is infected, it appears that lifelong parasitemia results. Many patients with chronic *T. cruzi* infection may be asymptomatic and thus could pass the blood donor medical questions [170]. The organisms can survive in refrigerated blood and can be transmitted by transfusion. Cases of transfusion-transmitted Chagas' disease are extremely rare in the United States [171, 172]. It is estimated that up to 100,000 infected Latin American immigrants live in the United States. In the Los Angeles area, which has a large Latin American population, about 1 in 1000–7500 and in Miami 1 in 9000 donors have antibodies to *T. cruzi* [173, 174]. Thus, transfusion-transmitted *T. cruzi* infection is a concern for the United States' blood supply [175, 176]. Antibody tests are currently being evaluated [177] including a questions designed to identify donors at risk combined with antibody testing.

Tick-borne diseases

Several tick-borne diseases have been recognized in the past 30 years and are becoming increasingly well understood. The infectious agents are transmitted to humans by tick bites and circulate in the blood. Therefore, transfusion transmission is possible [178].

Babesiosis

Babesia microti and *Babesia bovis* are protozoans that occasionally infect humans by tick bites. The protozoan causes an acute illness of fever,

malaise, and sometimes hemolytic anemia; however, many infected individuals are asymptomatic. Thus, *B. microti* can be transmitted by blood donated by asymptomatic infected donors [179–184]. The ticks are prevalent in the Northeast, mid-Atlantic, and upper Midwest. A serologic test is available, but it is not yet suitable for large-scale donor screening. Some geographic or seasonal defer individuals is not practical [185] and a history of a tick bite is not a good predictor of a positive serologic test [186]. Thus, currently there is no effective strategy to prevent transfusion-transmitted babesiosis.

Granulocyte anaplasmosis

This tick-borne disease previously called ehrlichiosis involves fever, headache, myalgia, and may be accompanied by thrombocytopenia, leukopenia, elevated liver enzymes, and severe forms involved pneumonia, renal failure, shock, and death. Transfusion transmission [187] and parenternal transmission have been documented [188]. No strategies are in place to prevent this transfusion-transmitted infection.

Lyme disease

Borrelia burgdorferi, a spirochete that is transmitted by ticks to humans, causes an acute illness of fever, malaise, and an erythematous annular spreading skin lesion followed by a chronic phase characterized by neurologic and/or cardiac symptoms with or without arthritis. However, in up to 40% of persons the infection is asymptomatic [189]. The spirochetes survive in stored blood for up to 45 days [190]. Thus, transmission of B. burgdorferi by transfusion is theoretically possible, but this has not been reported. Although a serologic test is available, it is not a suitable laboratory test for donor screening and the widespread prevalence of the tick makes it impractical to defer donors from endemic areas.

Rocky mountain spotted fever

Rocky mountain spotted fever (RMSF) is the most severe tick-borne disease, but only one case of transfusion-transmitted RMSF has been reported [178].

Leishmaniasis

Leishmaniasis, a protozoa disease endemic in the tropics, is transmitted to humans by the bite of a sand fly. Blood-borne transmission can occur and the parasites apparently survive in stored blood for up to 30 days [191].

15.7 Current issues with transfusion-transmitted Infections

Variant Creutzfeldt–Jakob disease and bovine spongiform encephalopathy

Reports from England of the possible association of a variant of Creutzfeldt–Jakob disease (CJD) with ingestion of beef from cows affected

with "mad cow disease" [192]. CJD has been transmitted by dura, corneas, and pituitary growth hormone from CJD patients and EEG electrodes used on CJD patients [193, 194] raised the question of whether the variant of CJD could be transmitted by blood transfusion [193, 195, 196]. CJD is a rare neurological disorder thought to be associated with a transmissible agent [194–196]. The agent may be an abnormally configured host protein called a prion. However, since the exact cause of CJD is not known, there is no laboratory test to identify the infectious agent if one exists.

It is clear that variant CJD is a human form of bovine spongiform encephalopathy (BSE) contracted by eating meat from cows with BSE [193, 194]. Since there is no test for the causative agent, studies involving experimental animal models have been used to show that blood appears to be infectious [195–200]. However, the timing, duration, and level of infectivity are difficult to establish. Fortunately, prions and infectivity do not seem to follow the factions of plasma used to prepare derivatives such as albumin, immune globulins, or coagulation factor concentrates [201, 202], and there is no increased incidence of variant Creutzfeldt–Jakob disease (vCJD) in hemophiliac patients exposed to coagulation factor concentrates [203].

Since the risk of transfusion-transmitted vCJD was not known (although though to be low to absent) and in the absence of a blood screening test the FDA required that individuals who have spent 6 months or more in the United Kingdom between 1980 and 1996 be deferred as blood donors. Subsequently four probable cases of transfusion-transmitted vCJD have been reported [204–207]. Another approach to determining transfusion transmissibility is look back in which the recipients of blood from donors subsequently found to have CJD are located to determine whether they have developed the disease. A donor developed CJD who had given 90 times over 35 years, but no cases of transfusion-transmitted vCJD were found [208]. It appears that there is a 14% likelihood of vCJD developing in recipients who survive 5 years or longer [209].

While some transfusion medicine professionals and policy makers believed that handling of possible transfusion-transmitted CJD was excessively conservative, others believe that these actions are appropriate given the public's fears of transfusion and the perception that the transfusion medicine community did not act as aggressively as it should have in the early days of the AIDS epidemic. Fortunately, thus far, transfusion transmission of spongiform encephalies does not appear to be a significant clinical problem.

15.8 Xenotropic murine leukemia virus-related virus

This is in the family of gammaretroviruses that cause leukemia or other syndromes in animal host species. Infection of humans with gammaretroviruses was identified in 2006 when the xenotropic murine leukemia virus-related virus (XMRV) virus was detected in some men with

localized prostate cancer. Subsequent studies have not concluded that the virus is related to prostate cancer but in 2009 a very strong statistical association between the XMRV virus and chronic fatigue syndrome (CFS) was reported [210]. The virus was present in 67% of CFS patients and only 3.7% of healthy controls. XMRV is infectious from either cell-associated or cell-free material from infected individuals [210]. Thus, concern has arisen regarding the possible transfusion-transmission of this agent. Those studies are underway at the time of this writing, but as a precautionary measure, a questioning strategy is being developed to defer from blood donation individuals who have been diagnosed with chronic fatigue syndrome since they may harbor the virus and thus potentially transmit it to blood recipients. The validity of the original work remains unsettled and so the XMRV situation is not clear.

15.9 Influenza

Concern about a possible influenza pandemic due to H1N1 raised the issue of possible transfusion transmission of this virus. Viremia apparently occurs in classic influenza but the incidence is low and short-lived [211]. However, a pandemic strain might be more pathogenic causing more concern about potential blood donation during an asymptomatic viremic phase. As part of pandemic planning, more extensive study of viremia in these patients is warranted.

15.10 Other diseases

Theoretically, any disease in which microbes circulate in the blood and survive for a few days in stored blood components could be transmitted by transfusion. This is even more of a potential if the initial disease is often asymptomatic or if a carrier state develops often. The diseases of most concern for transmission by blood transfusion have been discussed above. A few other diseases that are almost never transmitted by transfusions in the United States are toxoplasmosis, leishmaniasis, microfilaremia, and African trypanosomiasis. There is concern, however, that emerging pathogens, the mobility of people, and continued immigration can alter the situation with transfusion-transmitted diseases. Recent examples as described in this chapter are dengue, chikengunya, babesia, and anaplasma.

15.11 Introduction of new tests

As new transfusion-transmitted diseases are recognized or as transfusion transmission of presently known diseases becomes a greater factor, the issue of how to minimize disease transmission continues to be crucial [212]. As should be apparent from discussions in this chapter and in Chapters 3, 4, 8, and 10, it is not so simple as merely adding another

Table 15.5 Issues in considering new transfusion-transmissible disease screening tests.

"Because it's there"
Prevalence of agent in general population
Geographic distribution of agent
Value of medical history
Known high-risk groups
Infectivity of agent
Likelihood of disease
Nature of disease
Availability of therapy for disease
Quality of screening test
Availability of confirmatory test
Status of epidemic (stable; increasing)
Asymptomatic chronic phase
Potential for secondary spread
Cost

Source: Dodd RY. Scaling the heights. Transfusion 1995; 35:186–187.

screening test. This issue may ultimately be resolved by pathogen inactivation of blood products (Chapter 5), but methods for treating all components are not yet available. Several of the chapters cited emphasize that blood safety depends on many strategies, not just the laboratory test. The example of the effect of donor history in reducing the infectivity of the blood in San Francisco (Figure 15.3) is an excellent one, as well as the reduction in posttransfusion hepatitis from the change to volunteer instead of paid donors. In considering testing, some of the diseases may be regional or seasonal in nature (babesia), but with the mobility of the population, regional screening practices may not be suitable. However, this probably means that the screening tests will be very inefficient in many parts of the United States where the disease is not endemic. The prevalence of the disease, the infectivity of the agent, the status of the epidemic, and the likelihood of a carrier state are examples of factors about the infectious agent and the disease that can be taken into consideration when making a decision about a screening test (Table 15.5) [213]. In addition, the severity of the disease and the availability of treatment might be considered. However, when all of the scientific discussion is completed, there are the issues of social policy and public expectations of blood supply [213–215]. A report from the National Academy of Sciences [216] has been critical of the handling of the early days of the AIDS epidemic, although this report has been criticized as using hindsight and being inaccurate [217]. Nevertheless, the message from the public is that they expect the transfusion medicine community to take steps to achieve the maximum possible safety and dire consequences can result if those responsible do not respond [215]. To what extent should cost-effectiveness be a consideration in improving blood safety? Cost-effectiveness has been used in some situations but not others [218]. In the past, policy makers and politicians have shown little tolerance for failure to take steps that would decrease

risks to their constituents, regardless of the cost. A precautionary principle has been described in which lower level of proof of harm is used to establish policy if waiting for more definitive proof results in costly or irreversible harm [214]. A more rational approach has been sought by many; however, the decision-making process is still a complex one with no easily discernible structure for these decisions.

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16 The HLA System in Transfusion Medicine and Transplantation

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16.1 The HLA system

The human leukocyte antigen (HLA) system was first investigated in the 1950s by Dausset, Payne, and van Rood, who studied leukocyte-agglutinating antibodies as possible causes for autoimmune diseases and febrile transfusion reaction [1]. Leukoagglutinins were observed in sera from multiparous women and previously transfused patients. The first HLA antigen defined by a leukoagglutinin, called MAC, was later renamed HLA-A2.

Graft rejection was found to be associated with the development of antibodies against allogeneic leukocytes. The genetic loci involved in the rejection of foreign organs are known as the major histocompatibility complex (MHC), and highly polymorphic cell surface molecules are encoded by the MHC. The human MHC is called the HLA system because these antigens were first identified and characterized using alloantibodies against leukocytes.

The HLA system has been well known as transplantation antigens, but the primary biological role of HLA molecules is in the regulation of immune response [2].

Genomic organization of the human MHC

The human MHC maps to the short arm of chromosome 6 (6p21) and spans approximately 3600 kilobases of DNA [3]. The human MHC can be divided into three regions. The class I region is located at the telomeric end of the complex, the class II region at the centromeric end, and the class III region in the center.

The class I region consists of the three classical genes (*HLA-A*, *HLA-B*, *HLA-C*), the three nonclassical genes (*HLA-E*, *HLA-F*, *HLA-G*), and 12 noncoding genes or pseudogenes [3, 4]. The *HLA-A*, *HLA-B*, and *HLA-C* loci encode the heavy α chains of class I antigens. The expression of

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nonclassical class I genes are restricted and have limited polymorphism, and their functions are less well known.

The class II region consists of a series of subregions, each containing *A* and *B* genes encoding α and β chains, respectively [3, 4]. The *DR*, *DQ*, and *DP* subregions encode the major expressed products of the class II region. The *DR* gene family consists of a single *DRA* gene and nine *DRB* genes (*DRB1* to *DRB9*). Different HLA haplotypes contain particular numbers of *DRB* loci. The *DRB1*, *DRB3*, *DRB4*, and *DRB5* loci are usually expressed, and the other *DRB* loci are pseudogenes. The *DRA* locus encodes an invariable α chain and it binds various β chains. HLA-DR antigen specificities (i.e., DR1 to DR18) are determined by the polymorphic DRβ1 chains encoded *DRB1* alleles. The *DQ* and *DP* families each have one expressed gene for α and β chains and additional pseudogenes. The *DQA1* and *DQB1* gene products associate to form DQ molecules, and the *DPA1* and *DPB1* gene products form DP molecules. The nonclassical class II genes, *HLA-DO* and *HLA-DM*, may play a role during antigen processing and presentation.

The class III region does not encode HLA molecules but contains genes for the complement components (*C2*, *C4*, and *CFB*) and the cytokines (*TNF*, *LTA*, and *LTB*).

HLA haplotypes

HLA genes are closely linked, and the entire MHC is inherited as an HLA haplotype in a Mendelian fashion from each parent. The segregation of HLA haplotypes within a family can be assigned by family studies (Figure 16.1). Two siblings have a 25% chance of being genotypically HLA
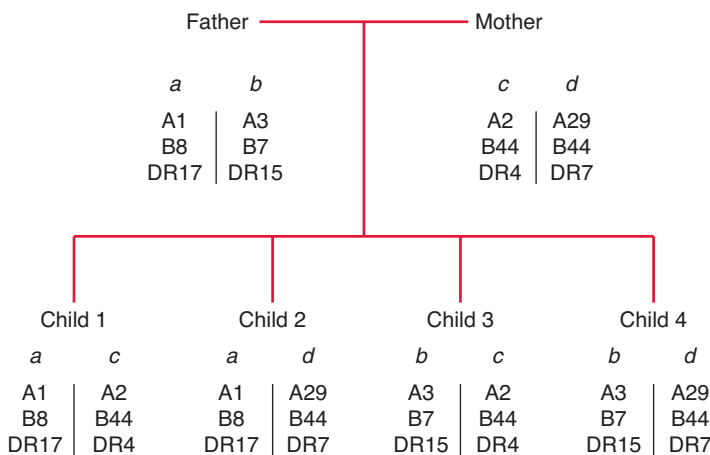
Figure 16.1 Mendelian inheritance of HLA haplotypes in an illustrated family. The parental HLA haplotypes can be inferred from phenotypes of the family members. The paternal HLA haplotypes are HLA-A1, B8, DR17 (haplotype "a")/HLA-A3, B7, DR15 ("b"); and the maternal HLA haplotypes are HLA-A2, B44, DR4 ("c")/HLA-A29, B44, DR7 ("d") (Courtesy of S. Yoon Choo).

identical, a 50% chance of being HLA haploidentical (sharing one haplotype), and a 25% chance that they share no HLA haplotypes. Recombination within the HLA system occurs with a frequency less than 1%, and it appears to occur most frequently between the *DQ* and *DP* loci.

Possible combinations of antigens from different HLA loci on a haplotype are enormous, but some HLA haplotypes are found more frequently than expected by chance in certain populations. This phenomenon is called the linkage disequilibrium. For example, *HLA-A1, B8, DR17* is the most common HLA haplotype among Caucasians, with a frequency of 5%.

The distribution and frequency of HLA antigens vary greatly among different ethnic groups. It has been postulated that this diversity of HLA polymorphism was derived and evolved by unique selective pressure in different geographic areas. This could be related to the role of the HLA molecule in the presentation of significant infectious agents in the different areas of the world.

Tissue expression of HLA

HLA class I molecules are expressed on the surface of almost all nucleated cells. They can also be found on red blood cells and platelets. Class I molecules on the mature red cell surface derive likely from endogenous synthesis by erythroid precursor cells and also from adsorption of soluble antigens present in plasma. The Bg red cell antigen phenotypes represent various HLA antigens. HLA class I molecules present on the platelet surface probably derive from megakaryocytes and also from adsorption of soluble antigens from plasma.

Class II molecules are expressed on B lymphocytes, antigen-presenting cells (APC) (monocytes, macrophages, and dendritic cells), and activated T lymphocytes.

Structure and polymorphism of HLA molecules

Class I molecules consist of glycosylated heavy chains of 44,000–45,000 daltons (44–45 kDa) encoded by the HLA class I genes and a noncovalently bound extracellular 12 kDa β_2-microglobulin (β_2m) [5] (Figure 16.2). Human β_2m is invariant and is encoded by a non-MHC gene located on chromosome 15. The class I heavy chain has three extracellular domains (α_1, α_2, and α_3), a transmembrane region, and an intracytoplasmic domain. Each extracellular domain comprises about 90 amino acids. The α_1 and α_2 domains contain variable amino acid sequences, and these domains determine the serologic specificities of the HLA class I antigens. Three-dimensional structures of the extracellular portion of several HLA class I molecules was revealed by X-ray crystallography [6] (Figure 16.3). The α_3 and β_2m domains form immunoglobulin constant domain-like folds (Figure 16.3A). The heavy chain α_1 and α_2 domains form a unique structure consisting of a platform of eight antiparallel β strands and two antiparallel α-helices on top of the platform (Figure 16.3B). A groove is formed by the two α-helices and the β-pleated floor, and this is the binding site for processed peptide antigen [2]. The class I peptide binding
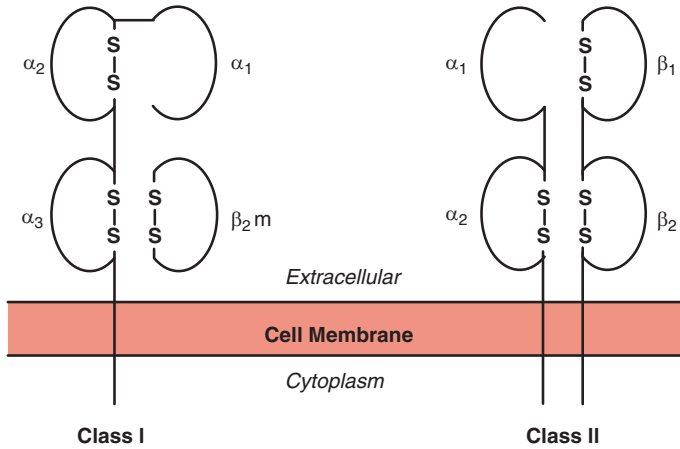
Figure 16.2 Schematic diagram of HLA class I and class II molecules. The class I molecule consists of a heavy α chain and a light chain β_2-microglobulin. The class II molecule is a heterodimer consisting of α and β chains. Disulfide bonds are denoted by –s–s–. (Reprinted by permission from Macmillan Publishers Ltd: Nature. Bjorkman PJ, Saper MA, Samraoui B, et al. The structure of the human class 1 Histocompatibility antigens, HLA-A2. Nature 1987;329:506–512.)
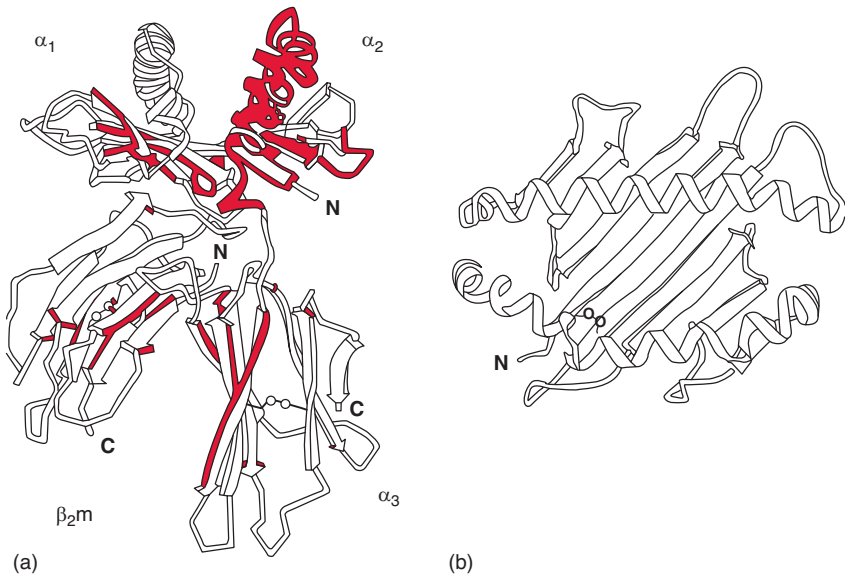
Figure 16.3 Schematic representation of the structure of HLA-A2 molecule. **(**a) Extracellular domains of HLA-A2. The polymorphic α_1 and α_2 domains are located at the top and the membrane proximal immunoglobulin-like domains (α_3 and β_2m) at the bottom. (b) The α_1 and α_2 domains form a platform with a single eight-stranded β-pleated sheet (shown as thick arrows), covered by two α-helices (represented as helical ribbons). Disulfide bonds are indicated as two connected spheres. N, amino terminus. (Reprinted by permission from Macmillan Publishers Ltd: Nature. Bjorkman PJ, Saper MA, Samraoui B, et al. The structure of the human class I histocompatibility antigen, HLA-A2. Nature 1987; 329:506–512.)

groove accommodates a processed peptide of 8–10 (predominantly nonamers) amino acid residues [7].

The products of the class II loci *DR, DQ,* and *DP* are heterodimers of two noncovalently associated glycosylated polypeptide chains: α (30–34 kDa) and β (26–29 kDa) (Figure 16.2). The difference in molecular weights of the two chains is primarily due to different glycosylation. The α and β chains are transmembrane and they have the same overall structures. An extracellular portion composed of two domains (α_1 and α_2, or β_1 and β_2) is anchored on the membrane by a short transmembrane region and a cytoplasmic domain. The extent of class II molecule variation depends on the subregion and the polypeptide chain. Most polymorphisms occur in the first amino terminal domain of *DRB1, DQB1,* and *DPB1* gene products. The three-dimensional structure of the HLA-DR molecule is similar to that of the class I molecule [8]. The α_2 and β_2 domains are similar to immunoglobulin constant domains. The α_1 and β_1 domains form an antigen-binding groove. The class II groove is more open so that longer peptides (12 amino acids or longer) can be accommodated [9].

The HLA system is known to be the most polymorphic in humans. The HLA polymorphism is not evenly spread throughout the molecule but is clustered in the antigen-binding groove [2, 5, 9]. Amino acid variations in several regions change the fine shape ("pockets") of the groove and thus the peptide-binding specificity of HLA molecules [10]. This is the structural basis for the binding specificity between HLA molecules and peptides that in turn determines the immune response.

Immunologic role of HLA molecules: peptide presentation

T cells recognize processed peptides on the cell surface. Zinkernagel and Doherty demonstrated in 1974 that T lymphocytes must have the same MHC molecules as the APC to induce immune response [11]. The phenomenon that peptides are bound to MHC molecules and these complexes are recognized on the cell surface by the T-cell receptor (TCR) is called the MHC restriction. During the T-cell maturation in the thymus, T lymphocytes are educated and selected to recognize the self-MHC molecules, and thereafter MHC molecules play a role as determinants of immune response.

The peptide-binding specificities of HLA molecules are determined by a relatively limited number of amino acid residues located in the peptide-binding pockets [12]. The fine structure of these pockets changes depending on the nature of the amino acids within the groove. Different HLA molecules show characteristic amino acid residue patterns in the bound peptides [10]. Amino acid residues that are located at particular positions of the peptides are thought to act as the peptide's anchoring residues in the peptide-binding groove.

The nature and source of peptides that will bind to class I or class II molecules are different [9, 13]. The mechanisms governing formation of MHC-peptide complexes are known as antigen processing [14]. Class I-restricted T cells recognize endogenous antigens synthesized within the

target cell, whereas class II-restricted T cells recognize exogenously derived antigens. Antigen processing involves degrading of the antigen into peptide fragments. Cellular or virus-induced proteins are processed by the cytoplasmic proteasome complex. Proteasomes are multisubunit proteinase complexes, and genes encoding components of the proteasomes are located within the class II region [3]. The resulting peptides are translocated into the endoplasmic reticulum (ER), and this process is mediated by the transporter associated with antigen processing (TAP). *TAP* genes are also located within the class II region. Within the ER, peptides associate with newly synthesized class I molecules. The peptide-bound class I molecules are transported to the cell surface via the Golgi apparatus, and the complex is recognized by the TCR of CD8+ lymphocytes.

Class II expression is mainly restricted to the APC, including B cells, monocytes/macrophages, dendritic cells, and Langerhans cells. Specialized APCs are capable of stimulating T-cell division. Class II molecules on the APC surface present exogenously derived antigens to CD4+ helper T cells [14]. Newly synthesized class II $\alpha\beta$ heterodimer chains are complexed to a 31-kDa polypeptide called the invariant I (Ii) chain in the ER. The Ii chain is encoded by a non-MHC gene and prevents binding of the $\alpha\beta$ heterodimers to peptides present in the ER. The class II $\alpha\beta$-Ii complex is transported through the Golgi complex to an acidic endosomal compartment, where the Ii chain is released. This dissociation of Ii from the $\alpha\beta$ heterodimer permits binding of peptides into the peptide-binding groove. Class II molecules are presenting antigens originated from an exogenous source. Extracellular exogenous proteins are endocytosed and undergo degradation in the acidic endosomal compartment. After the class II molecule-peptide complex is transported to the APC surface, the complex is recognized by the TCR of CD4+ lymphocytes.

CD1 molecules are a family of MHC class I homologs that bind β_2m and present lipids to T lymphocytes [15].

They resemble the classical peptide antigen-presenting MHC molecules except that the large, exclusively nonpolar and hydrophobic, antigen-binding groove of CD1 has evolved to present cellular and pathogen-derived lipid antigens.

The TCR is a molecule on the surface of T lymphocytes that recognizes HLA-peptide antigens. There are two forms of TCR: polypeptide heterodimers composed of either $\alpha\beta$ or $\gamma\delta$ [16]. The $\alpha\beta$ TCR is present on more than 95% of peripheral blood T cells. The TCR molecule is associated on the cell surface with a multichain accessory molecule CD3. The amino acid sequence variability of the TCR resides in the amino terminal domains of the α and β chains. During the recognition process between TCR and HLA-peptide complex, the signal from the T-cell complex is enhanced by simultaneous binding of HLA molecules by a specific coreceptor. The CD4 molecule on helper T cells interacts with a class II molecule on the APC, and the CD8 molecule on cytotoxic T cells interacts with a class I heavy chain on the target cells. Engagement of the TCR with HLA-peptide results in activation of T lymphocytes. Structural

studies show that the overall mechanism of TCR recognition of self MHC-peptide and allogeneic MHC molecules is similar [17].

Natural killer (NK) cells are a subset of lymphocytes (10–30% of peripheral blood lymphocytes (PBLs)) that lack both CD3 and TCR and exert cytotoxicity [18]. NK cell recognition is not MHC-restricted. NK cells have been known to recognize the loss of expression of HLA class I molecules (missing self) and destroy cells with decreased expression of class I molecules such as some tumors and virally infected cells. Other cells with normal MHC class I expression can still be NK targets if they provide appropriate signals to activating NK-cell receptors. Many different NK-cell receptors have been identified, and the majority of their ligands are HLA class I molecules. NK cells are regulated by both inhibitory and activating signals resulting from the NK cell receptor–ligand binding. Clinical studies utilizing the NK cell alloreactivity for antileukemia effect is explored in HLA mismatched stem cell transplants [19].

16.2 Clinical HLA testing

Laboratories involved in clinical HLA testing have been called by various names: tissue typing laboratory, HLA laboratory, histocompatibility laboratory, or clinical immunogenetics laboratory. HLA testing in the transplant workup includes HLA typing of the recipient and the potential donor, screening and identification of preformed HLA antibodies in the recipient, detection of preformed antibodies in the recipient that are reactive with lymphocytes of a prospective donor (crossmatch), and detection of de novo development of donor-specific anti-HLA antibodies after transplantation.

Serologic typing of HLA antigens

The complement-mediated microlymphocytotoxicity technique has been used as the standard for serologic typing of HLA class I and class II antigens [20, 21]. HLA typing sera are mainly obtained from multiparous alloimmunized women, and their HLA specificities are determined against a panel of cells with known HLA antigens in a process similar to red cell antibody identification. Some monoclonal antibody reagents are also used.

PBLs express HLA class I antigens and are used for the serologic typing of HLA-A, HLA-B, and HLA-C. HLA class II typing is done with isolated B lymphocytes because these cells express class II molecules. Different lymphocyte populations can be isolated by various techniques; for example, B lymphocytes can be isolated using B cell-specific monoclonal antibody-coated magnetic beads. HLA typing is performed in multiwell plastic trays with each well containing a serum of known HLA specificities. Lymphocytes are placed in the well and incubated, and complement is added to mediate the lysis of antibody-bound cells. The common source of complement is the rabbit. Cell lysis is detected by fluorescence dye staining.

When a serum shows reactivity with only one antigen, the antibody is called monospecific and the antigen specificity is called private. Some

Table 16.1 Numbers of recognized private HLA antigen specificities and alleles.

Locus	Antigen specificities	Alleles
HLA-A	24	1001
HLA-B	55	1605
HLA-C	9	690
HLA-DRB1	17	785
HLA-DQB1	7	108
Total	112	4189

http://www.ebi.ac.uk/imgt/hla/stats.html.

antibody molecules are reactive with two or more distinct antigens if the antigens share a same or similar antigenic determinant. Antigenic determinants shared by two or more antigens are called public specificities. Since the HLA polymorphism is formed by a patchwork of variable sequences shared by different antigens [5], public specificities are commonly encountered. Cross-reactivity is observed when a number of antigens share an antigenic determinant. A cluster of antigens sharing antigenic determinants are called a cross-reactive group.

Formal assignments of serologically defined antigens were given by the World Health Organization HLA Nomenclature Committee, which is responsible for the nomenclature of the HLA system [4]. HLA antigens are designated alphabetically by the locus and numerically by the order of official assignment. HLA-C antigens are given the designation Cw to avoid confusion with complement components. The naming of new HLA antigens had been discontinued, and now only newly discovered alleles are designated.

Molecular typing of HLA alleles

Studies of the HLA system using monoclonal antibodies, electrophoretic gel analysis, cellular assay, and molecular techniques have revealed that the extent of HLA polymorphism is far higher than previously known antigen specificities (Table 16.1). Serologically undistinguishable variants or subtypes of HLA class I antigens were identified by these methods, and it was demonstrated that these variants are different from the wild type by a few (usually one to five) amino acids, but these can be differentially recognized by alloreactive or MHC-restricted T lymphocytes [22]. Clinical molecular typing has been developed to distinguish serologically undistinguishable but functionally discrete HLA alleles.

Molecular typing of HLA class II genes began in the mid-1980s. The first technique used was a restriction fragment length polymorphism (RFLP) Southern blotting analysis. RFLP analysis is based on DNA sequence polymorphism that can be detected by restriction endonucleases recognizing and cutting specific DNA sequences. The limitation in the RFLP analysis is that the number of restriction sites recognized by a given enzyme is limited in the gene, and thus only a few selected sequence

polymorphisms are detected. Furthermore, detected DNA polymorphism may reside in the coding region or noncoding region, and the polymorphism in the latter region is not relevant. RFLP analysis has been phased out.

The polymerase chain reaction (PCR) using thermostable DNA polymerase, called Taq polymerase, became available to amplify and study HLA genes [23]. Synthetic oligonucleotide primers were designed to initiate DNA amplification of specific HLA genes. Resulting PCR products have been sequenced, and the accumulated data established the HLA allele sequence database [24]. PCR-based clinical HLA typing was first developed using sequence-specific oligonucleotide probe (SSOP) methods [25]. The hypervariable exon 2 sequences encoding the first amino terminal domains of the *DRB1*, *DQB1*, and *DPB1* genes are amplified from genomic DNA by PCR reaction. Based on the HLA sequence database, a panel of synthetic oligo-nucleotide sequences corresponding to variable regions of the gene are designed and used as SSOP in hybridization with the amplified PCR products. Alternatively, polymorphic DNA sequences can be used as amplification primers, and in this case only alleles containing sequences complementary to these primers will anneal to the primers and amplification will proceed. This strategy of DNA typing is called the sequence-specific primer method, and it detects sequence polymorphism at given areas by the presence of a particular amplified DNA fragment. High-resolution *HLA-DRB1* allele typing had become feasible to support unrelated donor marrow transplantation [26].

The development of HLA class I allele typing has been much behind that of class II. The class I polymorphism is located in the two domains, α_1 and α_2 (requiring amplification of two exons and an intervening intron), and there are many more polymorphic sequences (requiring more probes or primers than in class II). These differences made it more challenging to develop molecular typing strategies for class I.

Actual DNA sequencing of amplified products of multiple HLA loci, called sequence-based typing, is increasingly used as clinical HLA typing in unrelated donor stem cell transplantation.

HLA alleles are designated by the locus followed by an asterisk, a two-digit number corresponding to the antigen specificity, and the assigned allele number [4 For example, *HLA-A*02:10* represents the tenth *HLA-A2* allele encoding a unique amino acid sequence within the serologically defined HLA-A2 antigen family. A number of HLA alleles have been identified that are not expressed. Because of the existence of null HLA alleles, some believe that serological typing has to supplement DNA-based HLA typing [27].

Cellular assays

The mixed lymphocyte culture (MLC) test is performed by mixing peripheral blood mononuclear cells from two individuals in vitro and observing cellular proliferation. HLA class II disparity between individuals is responsible for the stimulation of lymphocytes. The one-way MLC test is performed by mixing responder T cells with stimulator cells irradiated to

prevent their proliferation. After 5 or 6 days, proliferative response of the responder's cells is indicated by increased DNA synthesis, conventionally by measuring incorporation of tritiated thymidine into the DNA. Reactive MLC results indicate *HLA-D* region incompatibility. The MLC technique had also been used to type cellularly defined HLA-D specificities using *HLA-D* region homozygous typing cells as stimulators. These cellular typing techniques for class II region compatibility had been replaced by the class II allele typing in the clinical laboratory [28].

Cell-mediated lympholysis or cytotoxic T lymphocyte assay can demonstrate the in vitro killing effect of sensitized T lymphocytes against allogeneic cells. The cytotoxic effect mainly represents HLA class I disparity. The target lymphocytes are radiolabeled with ^{51}Cr, and the cytotoxic effect is measured by the release of ^{51}Cr after culture of the two lymphocyte populations. Newer cellular function assays are being developed to assess the transplant recipient's pre- and posttransplant rejection risks [29], but it remains to be seen if any of these assays would prove to be clinically useful.

HLA antibody screening and lymphocyte crossmatch

Preformed HLA antibodies can be detected by testing the patient's serum against a panel of lymphocytes with known HLA specificities. The complement-dependent cytotoxicity (CDC) technique had been the standard, and the addition of antihuman globulin provides higher sensitivity. This test is called HLA antibody screening, and the results are expressed as the percentage of the panel cells that are reactive; this is called the percent panel reactive antibody (%PRA). For instance, if 10 of 40 different panel cells are reactive with a serum, the PRA is 25%. With a panel of well-selected cells representing various HLA antigens, antibody specificities can sometimes be assigned. Alternatively HLA antibody screening and identification can be performed by enzyme-linked immunosorbent assay (ELISA) and solid-phase tests (flow cytometry or Luminex). These technologies use purified or recombinant HLA molecules instead of live lymphocytes. Advantages include higher sensitivity/specificity and ability to detect noncomplement fixing antibodies as well. Solid-phase methods using single-antigen beads readily allow unambiguous determination of antibody specificities even in the patients with a high PRA. Calculated PRA (CPRA) can be measured by determining the specific HLA antibodies against incompatible antigens and the frequencies of these antigens in a given population [30]. When a potential donor becomes available, a final crossmatch is performed between the recipient's serum and the donor's lymphocytes to determine the compatibility. Lymphocyte crossmatching is done by CDC or more sensitive flow cytometry. The positive crossmatch results are generally predictive of the risks of graft rejection and shortened graft survival [31]. Antibodies to both HLA class I and class II antigens seem to be detrimental. The newly devised CPRA is important for the prospective organ transplant candidate to predict the chance of encountering an unacceptable antigen-positive and thus a crossmatch incompatible deceased donor. On the basis of this prediction, unnecessary positive

crossmatches with unacceptable antigen-positive donors can be reduced or avoided. Practice of CPRA-based "virtual crossmatching" in lieu of actual crossmatching, however, has limitations because the clinical importance of weak HLA antibodies has not been well understood and there is no perfect correlation between the strength of antibody and crossmatch results and clinical outcome.

Development of donor-specific antibodies against mismatched antigens after kidney transplantation has been shown to be associated with graft failure and thus posttransplant antibody monitoring using a highly sensitive method is suggested [32].

16.3 The human minor histocompatibility antigens

Minor histocompatibility antigens are processed peptides derived from normal cellular proteins that associate with self-HLA molecules [33]. Minor histocompatibility antigens are inherited and have allelic forms. The number of minor histocompatibility loci is probably high, and the antigenic difference is typically the result of a single nucleotide polymorphisms giving rise to a single amino acid substitution. Minor histocompatibility antigens have been defined by both class I and class II MHC-restricted T cells. Examples include the male-specific H-Y antigens and a series of HA antigens.

Minor histocompatibility antigen disparity can be associated with graft-versus-host disease (GVHD) in HLA-identical transplants (e.g., H-Y antigen in a male recipient and a female donor who has been immunized by pregnancy) [34]. Minor histocompatibility disparity can also result in a favorable graft-versus-leukemia (GVL) effect. Whether minor histocompatibility antigen disparity can have a significant impact as a risk (graft rejection, GVHD) or a benefit (GVL) factor might depend on the tissue-specific expression of proteins, the frequency of allelic forms, and the immunogenicity of peptides. Minor histocompatibility antigens as allogeneic target molecules may offer a tool for tumor-specific immunotherapy [35].

16.4 The HLA system and transplantation

HLA-A, HLA-B, and HLA-DR have long been known as major transplantation antigens. The role of HLA-C and other class II molecules has been recently investigated especially in hematopoietic stem cell transplantation.

Both T-cell and B-cell (antibody) immune responses are important in graft rejection [36]. T lymphocytes recognize donor-derived peptides in association with the HLA molecules on the graft. The graft may present different allelic forms of the minor histocompatibility antigens, and the donor's HLA molecules may present a different set of peptides to the

recipient's T cells. CD4+ T helper cells are activated by APCs carrying HLA class II molecules. The APCs from either the donor or the recipient can activate the recipient's T cells. The donor's APCs present in the graft cause the "direct" activation of the recipient's T helper cells. The recipient's APCs can acquire alloantigens that are shed from the graft, process into peptides, and present to T helper cells to cause the "indirect" activation. Direct T-cell allorecognition plays an important role in acute rejection and indirect T-cell allorecognition in late onset chronic rejection. Multiple cytokines including interleukins, tumor necrosis factor, and interferon are involved in the rejection response.

Antibodies to the graft fix complement and cause damage to the vascular endothelium, resulting in thrombosis, platelet aggregation, and hemorrhage. Hyperacute rejection occurs in patients who already have antibodies specific to a graft. Antibodies against ABO blood group and preformed HLA antibodies induce hyperacute rejection. HLA alloimmunization can be induced by blood transfusions, pregnancies, or failed transplants. Hyperacute rejection can be avoided in most cases by ABO-identical or ABO-major compatible transplantation and by confirming negative lymphocyte crossmatching. Acute rejection is primarily the result of T cell-mediated response. Chronic rejection may be due to antibody- and cell-mediated responses.

Solid organ transplantation

Solid organs can be donated by deceased donors, living related donors, or living unrelated donors. Deceased donors are brain dead but are maintained hemodynamically stable until the time of organ harvest. More than one-third of transplanted kidneys nationwide are from living related donors: siblings, parents, or grown-up children. Other organs from living donors are liver (segmentectomy or lobectomy), lung (segmentectomy or lobectomy), pancreas (hemipancreatectomy), or small intestine. Transplant success can be measured by several factors: rejection, graft survival, half-life of allograft, and patient survival.

The United Network for Organ Sharing (UNOS) administers deceased donor organ procurement and allocation, and monitors national policies for solid organ transplantation. Evaluation of potential donors is done by taking the donor's history and performing a physical examination and laboratory testing for infectious disease markers, organ function, and HLA typing with samples of peripheral blood, lymph nodes, or spleen. There are general and organ-specific criteria for the acceptance of donors, and the final decision on the suitability of donor organs is made by the transplantation programs. Local or regional organ procurement organizations are responsible for acquiring and allocating solid organs. Increasing numbers of patients are waiting for deceased donor organs, and the national waiting list on the UNOS as of June 2010 exceeds 108,000 [37]. The UNOS has developed separate allocation policies for different types of solid organ.

All potential transplant candidates are registered with the UNOS. In general, each patient is HLA typed and evaluated for various clinical

conditions, and each individual is given a numeric point. When a deceased donor becomes available, HLA typing is performed, and the results are compared with those of the waiting patients on the national list. When a six-antigen (HLA-A, B, and DR) matched patient is identified, the kidney is offered to that patient. Cases of zero mismatched HLA antigen in the rejection direction (i.e., donors homozygous for HLA antigens and recipients heterozygous) are considered the same as an antigen match. This mandatory sharing policy of zero-antigen-mismatched kidneys has been implemented on the basis of clinical experience showing the best transplant results in these categories [38]. When there is no such "perfect" match, the kidneys are usually offered to patients with the highest points in the local organ procurement area. The UNOS algorithm for allocating kidneys takes into account the time of waiting, quality of HLA mismatches between donor and recipient, CPRA > 80%, age (pediatric candidates are given extra points), and previous organ donation. Prospective HLA crossmatching is mandatory for kidneys and pancreas.

Allocation of livers and hearts is based primarily on medical urgency. Each liver transplant candidate is assigned a status code or probability of candidate death derived from a mortality risk score corresponding to the degree of medical urgency. Mortality risk scores are determined by the prognostic factors (serum creatinine, bilirubin, and INR) and calculated in accordance with the Model for End-Stage Liver Disease Scoring System and Pediatric End-Stage Liver Disease Scoring System.

Other factors influencing the allocation of organs include the preservation time and the size of the organ. Organ preservation is important to prevent significant ischemic injuries. Organs are allocated first within the area, then within the UNOS region, and finally at the national level. The complex system of solid organ procurement and allocation was developed to increase the supply of organs and ensure their equitable distribution under conditions of limited donor organ supply.

In solid organ transplantation, the blood group ABO system is the most important major histocompatibility antigen. Preexisting anti-A and anti-B antibodies cause hyperacute rejection because ABO antigens are expressed on endothelial cells in the allograft. Preformed HLA antibodies also cause hyperacute rejection. The problem of hyperacute rejection can be predicted by a positive donor lymphocyte crossmatch and can be avoided when transplantation is performed from a donor whose lymphocytes are not reactive with recipient's serum. The presence in the recipient of preformed HLA antibodies reactive with a donor's lymphocytes is generally a contraindication to kidney transplantation [31]. Desensitization protocols involving plasma exchange, intravenous immunoglobulin, and rituximab have been developed to decrease the levels of HLA or ABO antibodies so that kidneys can be transplanted across otherwise incompatible barriers in some cases [39, 40]. For heart transplant candidates, initial HLA antibody screening is routine and prospective lymphocyte crossmatching is usually performed only for the HLA alloimmunized patients. Pretransplant crossmatching is not

performed prior to liver transplants because of the urgent need of organs and the uncertain benefits of a crossmatch-negative transplant.

The benefits of HLA matching are well established in kidney transplantation. There is a clear relationship between the degree of HLA matching and kidney graft survival in transplants from living related donors [41]. Better results are obtained from an HLA-identical sibling donor than with HLA-haploidentical parents, siblings, or children. Kidney transplantation from a living unrelated donor shows graft survival superior to deceased donor transplantation (except for zero mismatched) despite a greater degree of HLA mismatch [42]. These favorable results are probably the result of shorter ischemic time and less renal damage. The influence of HLA matching on the survival of liver and thoracic organs is yet uncertain.

Immunomodulatory effect of transfusion

Red cell transfusions had been given to end-stage renal failure patients with chronic anemia. In spite of a risk of developing alloimmunization against HLA, some transfused patients had better allograft survival [43]. The beneficial effect of pretransplant blood transfusion was the basis for the implementation of a protocol of donor-specific transfusion (DST) prior to kidney transplantation from a living related donor in an attempt to induce tolerance while waiting for a transplant. The beneficial effect of pretransplant blood transfusion was also documented in patients receiving transfusions from random donors [44].

DST carries a risk of sensitization of the patients, and some do indeed develop antibodies against HLA antigens and thus cannot receive the planned transplant. Since cyclosporine was introduced as an immunosuppressive drug, it significantly increased graft survival and then no significant differences were found in the graft survival rates between the transfused and nontransfused recipients of deceased donor kidneys [45]. The use of recombinant human erythropoietin reduced or eliminated the need for red cell transfusions, and as a result, the primary HLA sensitization in patients with renal failure has significantly diminished.

Immunomodulatory mechanisms of transfusion are not clearly understood, but the effect could be due to multiple factors [46]. Probably leukocytes contained in the red cell components are likely effectors. A few reports suggest that combined kidney and hematopoietic cell infusion can induce mixed hematopoietic chimerism and immune tolerance [47].

Allogeneic hematopoietic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation is used to treat hematologic malignancy, severe aplastic anemia, severe congenital immunodeficiencies, and selected inherited metabolic diseases [48]. The source of hematopoietic stem cells had traditionally been bone marrow, but currently mobilized peripheral blood stem cells and umbilical cord blood are generally preferred.

ABO blood group incompatibility is not a clinically significant barrier to hematopoietic stem cell transplantation. The HLA system is the major histocompatibility antigen in stem cell transplants, and the HLA matching

is predictive of the clinical outcome. HLA mismatch between a recipient and a donor represents a risk factor not only for graft rejection but also for acute GVHD because immunocompetent donor T cells are introduced to the recipient. T-cell depletion of donor marrow results in lower incidence of acute GVHD but higher incidence of graft failure, graft rejection, malignancy relapse (loss of the GVL effect), impaired immune reconstitution, and later complication of Epstein–Barr virus-associated lymphoproliferative disorders [49, 50].

The risk of graft rejection or failure was especially high in patients with severe aplastic anemia because these patients were frequently alloimmunized by multiple blood transfusions prior to transplant and their preconditioning regimen is less intensive than that for leukemia [51].

The best compatible stem cells are from an identical twin or a genotypically HLA-identical sibling. An HLA-identical sibling is found in approximately 25% of patients. For those who do not have a matched sibling, an alternative related family member who is HLA haploidentical and partially mismatched for the nonshared HLA haplotypes may serve as a donor, but these transplants have a higher risk of developing acute GVHD and graft rejection or failure [52].

When an HLA-matched or partially mismatched suitable related donor is not available, phenotypically matched unrelated donors can be considered [53]. The National Marrow Donor Program (NMDP) was founded in the United States in 1986 to establish a volunteer marrow donor registry and to serve as a source of HLA-matched unrelated marrow donors [54, 55]. The chance of finding an HLA-matched unrelated donor depends on the patient's HLA phenotype [56]. Since there is high diversity of HLA polymorphism among different race groups, there is a different chance of finding a match within different race groups. The NMDP registry, called BE THE MATCH, now contains more than 8 million HLA-typed donors. More than 115,000 cord blood units are available for transplantation through the NMDP. There are also international donor registries in other countries, and most of these registries share their donors. The NMDP coordinated more than 4500 hematopoietic stem cell transplants in 2009.

Unrelated donor transplants are associated with an increased incidence of acute GVHD and graft failure/rejection compared with HLA-matched sibling transplants. Such an increase results partly from mismatch in HLA alleles and from minor histocompatibility antigens [57]. For this reason, *HLA-A, B, C,* and *DRB1* allele matching is strongly recommended for unrelated donor blood and marrow transplantation [58]. Some patients do not find a perfectly allele-matched (8/8 match for *HLA-A, B, C,* and *DRB1* alleles) or one allele-mismatched (7/8 match) acceptable unrelated donor. For these patients, cord blood could be considered as an alternative source of hematopoietic stem cells [59, 60]. Cord blood has an advantage that HLA mismatching is better tolerated. Customarily, up to two mismatches out of six HLA-A, B antigens and *DRB1* alleles are allowed. With this relaxed HLA matching criteria, almost all patients will find a suitably matched cord blood unit. To overcome the disadvantage of smaller stem cell numbers in

cord blood resulting in graft failure or delayed engraftment, double cord blood transplantation is often performed especially for adult patients.

Transfusion practice in stem cell transplantation

Transfusion policy should include measures to prevent alloimmunization in all potential stem cell transplant candidates. All transplant candidates and recipients should receive leukoreduced cellular components in order to prevent or reduce the risk of HLA alloimmunization. Transfusion from blood relatives should be avoided for a patient who is a candidate for a related donor stem cell transplant. The minor histocompatibility antigens are inherited independently of the MHC region, and thus any transfusions from blood relatives could lead to an exposure to possibly relevant minor histocompatibility antigens also present on the donor.

16.5 The HLA system in transfusion therapy

The HLA system can cause adverse immunologic effects in transfusion therapy. These effects are primarily caused by donor "passenger" leukocytes contained in the cellular blood components. HLA antibodies can be induced from previous alloimmunizing episodes and can cause platelet transfusion immune refractoriness, febrile transfusion reaction, and transfusion-related acute lung injury (TRALI).

HLA alloimmunization

Multiparous women are frequently alloimmunized to HLA [61], and their HLA antibodies may persist or become gradually undetectable. Primary HLA alloimmunization by blood transfusion is caused by the leukocytes in the cellular blood products (i.e., direct HLA alloimmunization) [62]. HLA antibodies found in alloimmunized patients usually show multiple specificities [63]. It is more common to find broadly reactive antibodies instead of multiple antibodies of private specificities in patients with high PRA.

The incidence of HLA alloimmunization following transfusions can vary with the patient's diagnosis and therapy [64]. HLA antibodies can be detected in 25–30% of transfused leukemic patients and can be presented in as high as 80% of aplastic anemia patients. Leukemic patients are usually transfused while receiving intensive chemotherapy, which induces immunosuppression and this reduces the incidence of transfusion-induced alloimmunization. Severe aplastic anemia patients who had received blood transfusions historically had a higher incidence of graft rejection following stem cell transplantations [65]. The prevalence of HLA antibodies among previously transfused healthy blood donors seems to be negligible [61].

Leukocyte reduction to less than 5×10 [6] can prevent or reduce the development of primary HLA alloimmunization [66]. The incidence of HLA antibody development, however, is not decreased or delayed by the

leukocyte reduction filtration in patients with previous pregnancies [67]. It appears that the secondary HLA immune response cannot be prevented by the degree of leukocyte reduction currently available.

Refractoriness to platelet transfusion

Platelet refractoriness is a consistently inadequate response to platelet transfusions (see Chapter 11). There are immune and nonimmune causes for poor posttransfusion increments [68, 69]. The major nonimmune adverse factors are fever, splenomegaly/hypersplenism, antibiotics (amphotericin B, vancomycin, ciprofloxacin), disseminated intravascular coagulation, infection, sepsis, stem cell transplantation, venoocclusive disease, and bleeding at the time of transfusion.

HLA class I antigens are expressed on platelets and the development of antibodies to HLA or platelet-specific antigens can cause immune destruction of transfused incompatible platelets, resulting in a refractoriness to random donor platelet transfusions. Diagnosis of platelet immune refractoriness is supported if antibodies against HLA and/or platelet-specific antigens are detected and nonimmune causes of platelet refractoriness are ruled out. In reality, most patients with refractoriness are found to have one or more concurrent nonimmune adverse factors. When patients are suspected for immune refractoriness, HLA and platelet-specific antibody screening is performed. Presence of HLA and/or platelet-specific antibodies can be detected by using various techniques including lymphocytotoxicity test, ELISA, flow cytometry, Luminex, monoclonal antibody-specific immobilization of platelet antigens assay, mixed passive hemagglutination assay, and solid phase assay [70]. Most refractory patients are immunized to HLA, and immunization to platelet-specific antigens is much less frequent.

Once the clinical and laboratory diagnosis of immune refractoriness is made, the use of special platelet products is indicated. Most patients who are refractory to random donor platelets because of HLA antibodies respond to HLA-matched or -selected platelets [68]. Some regional blood centers maintain large pools (several thousands or more) of HLA-A, B typed volunteer platelet apheresis donors. Patients with common HLA types will have a higher chance of receiving platelets from HLA-matched donors. For many recipients who require frequent platelet transfusions, it is not possible to find enough matched donors. Time constraint and donor's unavailability can also be problematic. It takes more than 2 days to recruit donors, to collect platelets, and to initiate bacterial sterility tests and complete infectious disease marker screening. HLA-matched siblings or HLA-haploidentical family members can donate platelets, but to prevent alloimmunization to minor histocompatibility antigens, these blood-related donors should not support patients who are candidates for a related donor stem cell transplant. If the specificity of the patient's antibodies can be determined, donors who are negative for corresponding HLA antigens can be selected. Prospective crossmatching of the patient's serum against the platelet donor's lymphocytes can also be used to identify compatible donors, but this approach is rarely used [71]. Community

blood donors who are mismatched with the patients for cross-reactive HLA antigens can also be tried, but most of the benefit derives from platelets well matched for HLA. Donors who are not perfectly matched with the patients, but homozygous for a given HLA locus can also be used (e.g., patient HLA-A2, 3 and donor HLA-A2 only).

A number of techniques have been tried to determine platelet compatibility [70]. Platelet crossmatching using a solid-phase red cell adherence technique has been developed [72]. This technique will detect platelet antibodies against HLA class I and platelet-specific antigens. Collected platelet apheresis units are crossmatched with the patient's serum, and crossmatch compatible units are identified. The efficacy of crossmatched platelets may be as good as HLA-matched platelets in some patients [71]. The main advantage of using platelet-crossmatched products over HLA-matched platelets is that these units are immediately available for transfusion.

Since primary HLA alloimmunization caused by platelet transfusion is induced by contaminating leukocytes [62], this potential problem and platelet refractoriness can be prevented or reduced by the use of the third-generation leukoreduction filter [73]. Prevention of alloimmunization is indicated for patients who are expected to need long-term platelet transfusions. In patients with previous pregnancies, leukocyte reduction does not reduce the incidence of HLA antibody development and platelet refractoriness [67]. Most previously pregnant patients appear to develop HLA antibodies by a secondary immune response during transfusion therapy. Platelets per se, soluble HLA antigens, residual leukocytes, or leukocyte fragments escaping leukoreduction filtration may be able to induce a secondary HLA immune response. Experience of the universal prestorage leukoreduction demonstrated decreased incidence of alloimmune platelet transfusion refractoriness [74].

Ultraviolet B (UVB) irradiation can reduce primary HLA alloimmunization74 [75]. UVB irradiation interferes with the function of APC, thus preventing the alloantigen presentation to the recipient's T helper cells.

Transfusion-associated graft-versus-host disease

When functionally competent allogeneic T lymphocytes are transfused into an individual who is severely immunocompromised, these T lymphocytes are not removed and can mount an immune attack against the recipient, causing transfusion-associated graft-versus-host disease (TA-GVHD) (see also Chapters 11 and 14). TA-GVHD is not common, and typically occurs in patients with congenital or acquired immunodeficiencies or immunosuppression that affects T lymphocytes.

TA-GVHD has also occurred in patients without apparent evidence of immunodeficiency or immunosuppression [76]. The majority of these studied cases involved a blood donor who was homozygous for one or more HLA loci for which the recipient was heterozygous for the same antigen [77]. This relationship can be called a one-way HLA mismatch in the GVHD direction and a one-way HLA match in the rejection direction.

As a result, the donor's cells will not be recognized as foreign by the recipient's lymphocytes, while the donor's lymphocytes will recognize HLA alloantigens present in the recipient. Other risk factors that appear to predispose to TA-GVHD in immunocompetent patients possibly include fresh blood, donation from blood-related donors, and Japanese heritage, although the latter two factors probably reflect the HLA homozygous donor [77]. Many affected patients received transfusions of freshly donated cellular blood products. Fresh blood contains larger numbers of viable and presumably competent lymphocytes than stored blood. The minimum number of viable donor lymphocytes required to mediate TA-GVHD is unknown. The one-way match more likely occurs when an HLA haplotype is shared by a donor and a recipient (HLA haploidentical), such as in directed donation from blood relatives and among populations with relatively homogeneous HLA phenotypes [77]. The latter possibility may account for the observation that more cases of TA-GVHD have been reported among Japanese patients.

The clinical features of TA-GVHD are similar to those of acute GVHD following a hematopoietic stem cell transplant, i.e., fever, rash, diarrhea, and liver dysfunction. TA-GVHD is further characterized by prominent pancytopenia due to marrow aplasia. Demonstration of donor-derived lymphocytes in the circulation of a patient with characteristic clinical findings is diagnostic for TA-GVHD. The persistence of donor lymphocytes can be tested by molecular HLA typing, by fluorescent in situ hybridization analysis using sex chromosome probes if donor and patient are of different sexes, and by VNTR/STR polymorphisms. The demonstration of donor-derived lymphocytes in a transfusion recipient is not diagnostic of TA-GVHD per se because donor lymphocytes can be normally detected in the recipient's circulation a few days after transfusion.

There is no effective treatment for TA-GVHD, and most affected patients die within 3 weeks from complications of infections and hemorrhage. Rare survivors may develop chronic GVHD.

The primary emphasis in TA-GVHD is prevention [78]. Gamma irradiation of cellular blood products with 25 Gy is the effective way of inactivating donor lymphocytes. Irradiation is indicated for susceptible patients with various conditions (e.g., congenital immunodeficiencies, hematopoietic stem cell transplants, and hematologic malignancies undergoing chemotherapy) and for patients receiving intrauterine transfusion, HLA-matched platelets, or blood components donated from a blood relative. Solid organ transplant recipients under immunosuppressive therapy and patients undergoing chemotherapy and radiation therapy for solid tumors probably do not require irradiated blood products [79]. TA-GVHD has not been observed in patients with acquired immunodeficiency syndrome.

UVB irradiation may become an alternative way to inactivate lymphocytes in blood components. UVB irradiation has a potential of preventing both TA-GVHD and HLA alloimmunization [75]. Pathogen inactivation technologies also prevent TA-GVHD (see Chapters 5 and 14).

Depletion of lymphocytes from blood products using the currently available leukoreduction filter is not effective in preventing TA-GVHD.

GVHD can also occur following transplantation of solid organs, especially livers. It is mediated by passenger T lymphocytes present in the transplanted organ. Similar to TA-GVHD, one-way HLA matching has been observed [80]. In the case of liver transplant-associated GVHD, the liver is spared because the offending passenger T lymphocytes recognize it as "self."

Febrile nonhemolytic transfusion reaction (see also Chapter 14)

Febrile nonhemolytic transfusion reaction (FNHTR) is defined as a temperature rise of more than 1°C or 2°F during or shortly after the transfusion. Fever can be accompanied by chills, and chills in the absence of fever can be considered as a mild febrile reaction. Fever and chills are the most common transfusion reactions, observed in up to 5% of transfused patients.

FNHTR is caused by either an interaction between the recipient's anti-leukocyte antibodies (usually anti-HLA and less commonly neutrophil specific) and donor leukocytes contained in the blood components or pyrogenic cytokines present in the blood components. Alloimmunization to leukocytes occurs in previously pregnant or multiply transfused patients, and they are at higher risk of developing the reaction. Febrile reactions to platelet transfusions may be associated with alloimmunization and poor posttransfusion platelet recoveries. FNHTR is more frequently associated with transfusions of platelets stored for more than 3 days. A number of cytokines—TNF-α, IL-1β, IL-6, and IL-8—are released from leukocytes during the storage of platelets at room temperature, and these cytokines have pyrogenic effects [81, 82].

Repeated FNHTR occurs in approximately 15% of transfusions, and thus premedication with an antipyretic is usually not considered for patients after a first reaction. Patients with underlying fever appear to be at high risk of developing FNHTR. Premedication with acetaminophen is widely used to prevent FNHTR, but it can still occur. Leukoreduction of stored platelets is not effective to prevent a febrile reaction, but prestorage leukoreduced platelets have reduced cytokine release during storage and less frequently cause FNHTR [83, 84]. Fresh platelet products with lower amounts of pyrogenic cytokines may be preferred for patients with repeated febrile reactions. Slower infusion of blood products can be helpful in preventing the febrile reaction. FNHTR is treated with acetaminophen and severe shaking chills are treated with meperidine.

Granulocyte transfusion

Renewed interest in granulocyte transfusion therapy has been generated by the feasibility of collecting therapeutic doses of cells using granulocyte colony-stimulating factor (see Chapters 7 and 11) [85]. There is no

effective pretransfusion compatibility test for granulocytes. HLA matching or prospective lymphocyte crossmatching may be recommended if the patient is known to be HLA alloimmunized because alloantibodies can limit the effectiveness of granulocyte transfusion and cause a severe febrile transfusion reaction [86]. Granulocyte transfusion can induce HLA alloimmunization, which in turn will decrease future transfusion effectiveness. Granulocyte concentrates also contain other white blood cells and significant numbers of platelets and RBCs. Granulocyte products contain high numbers of lymphocytes and thus should be gamma irradiated to prevent TA-GVHD if they are to be given to a susceptible recipient. Because of the significant amounts of RBCs, granulocytes should be obtained from an ABO major compatible donor and red cell crossmatch should be tested with the recipient's serum. Cytomegalovirus (CMV) seronegative donors are preferred to prevent transfusion-transmitted CMV infection for neonates and CMV seronegative patients at risk of developing serious CMV disease.

Transfusion-related acute lung injury

TRALI is a rare complication of transfusion resulting in noncardiogenic pulmonary edema (see Chapter 14) [87]. TRALI is characterized by acute respiratory distress, bilateral pulmonary edema, and severe hypoxemia. Fever and hypotension may be present. Chest X-ray reveals bilateral pulmonary infiltrates. TRALI is caused by antibodies against HLA (both class I and class II) or granulocyte-specific antigens [88]. Implicated antibodies are usually found in the plasma of transfused blood components. Intravenous immunoglobulin (IVIG) has also been implicated in TRALI. The antigen–antibody reaction probably activates complement, resulting in neutrophil aggregation and sequestration in the lungs. The release of neutrophil granules leads to pulmonary vascular damage and extravasation of fluid into the alveoli and interstitium. Demonstration of the HLA or granulocyte specificity of the donor's antibody against the patient's HLA or granulocyte antigen is direct laboratory evidence further supporting the clinical diagnosis of TRALI. Less commonly, implicated antibodies are found in the recipient. Alternative pathogenesis of TRALI includes the possible role of biologically active lipids produced during blood storage [89a, 89b].

Strategies of reducing the risk of TRALI include the preferential use of mostly male donor plasma products, and this practice has shown to be effective in the United Kingdom [90] and a similar approach has been implemented in the United States [91]. All blood donors implicated with a TRALI case should be deferred from the preparation of plasma-containing blood products.

Leukocyte depletion can be helpful in preventing repeat TRALI reactions when the recipient's antibodies were responsible, but it is not helpful if the antibodies are from the blood donor. Solvent-detergent treated plasma used in the European countries has not been associated with TRALI, but such products are not available in the States.

Neonatal alloimmune thrombocytopenia

Neonatal alloimmune thrombocytopenia (NAIT) develops as a result of maternal sensitization to paternally inherited platelet antigens in the fetus. Antiplatelet IgG antibodies cross the placenta and cause fetal and neonatal immune thrombocytopenia. About half of cases involve the firstborn. The most commonly involved platelet antigen is HPA-1a [92]. Platelet-specific antigens are generally weak immunogens, and genetic factors may influence whether HPA-1a-negative women will develop anti-HPA-1a antibody. Individuals with certain HLA haplotypes with *HLA-DRB3∗0101* allele are more likely to develop antibodies against HPA-1a antigen [93].

It has been thought that NAIT was caused only by antibodies against platelet-specific antigens. However, several case reports suggested that HLA class I antibodies may occasionally be involved [94].

HLA-disease association

Certain diseases, especially of autoimmune nature, are associated with particular HLA types [3]. The association level, however, varies among diseases and there is generally a lack of a strong concordance between the HLA type and the disease. The exact mechanisms underlying many HLA-disease associations are not well known, and other genetic and environmental factors may play roles as well.

Among the most prominent associations are ankylosing spondylitis with *HLA-B27*, narcolepsy with *HLA-DQB1∗0602/HLA-DRB1∗1501*, and celiac disease with *HLA-DQB1∗02*. The *HLA-A1, B8, DR17* haplotype is frequently involved in autoimmune disorders. Rheumatoid arthritis is associated with a particular sequence of the amino acid positions 66–75 in the DRβ1 chain that is common to the major subtypes of DR4 and DR1. Type I diabetes mellitus is associated with DR3,4 heterozygotes, and the absence of asparagine at position 57 on the DQβ1 chain appears to render susceptibility to this disease.

Primary or hereditary hemochromatosis (HHC) is one of the most common inherited diseases manifested by an increased absorption of dietary iron, resulting in excess iron deposition in the liver, heart, and endocrine organs and finally organ failure. This disease is caused by an autosomal recessive gene, and up to 10% of the population are heterozygous (carriers) and 0.5% homozygous. Previously the unidentified disease gene had been postulated to be closely linked to the *HLA-A* locus, especially on the *HLA-A3* haplotype [95]. Nonclassical *HLA-H* (later defined as *HFE*) was identified as a responsible gene for HHC [96]. *HLA-H* is located approximately 5 megabases telomeric to the *HLA-A* locus.

Severe hypersensitivity reaction occurs in 5% of HIV/AIDS patients receiving avacavir, a nucleoside analog reverse transcriptase inhibitor, therapy. *HLA-B∗5701* allele was found to be strongly associated with hypersensitivity. [97]. Introduction of pharmacogenetic *HLA-B∗5701* screening prior to avacavir therapy has reduced the prevalence of hypersensitivity.

Parentage HLA testing

In parentage testing, genetic markers of a child, biological mother, and alleged father are compared to determine exclusion or nonexclusion of the alleged father. There are some advantages of using HLA types in parentage testing. The HLA system is inherited in a Mendelian manner and is highly polymorphic; its recombination rate is low; mutation has not been observed in family studies; and antigen frequencies are known for many different ethnic groups. The HLA system, however, does not provide a high exclusion probability when the case involves a paternal HLA haplotype that is common in the particular ethnic group. Molecular techniques using non-HLA genetic systems are widely used for this purpose.

16.6 Conclusion and summary

The human MHC HLA is located on the short arm of chromosome 6. It is known to be the most polymorphic genetic system in humans. The biological role of the HLA class I and class II molecules is to present processed peptide antigens and thus determine the immune response. The HLA system is clinically important as transplantation antigens. Molecular HLA allele typing is used to provide HLA class I and class II allele matching in unrelated donor hematopoietic stem cell transplantation. Precise determination of preformed HLA antibodies and prospective lymphocyte crossmatching is critical in solid organ transplantation to prevent allograft rejection. HLA alloimmunization causes various problems in transfusion therapy. The HLA system is associated with certain diseases by direct involvement of the HLA molecules, more frequently by a closely linked susceptibility gene, or by unknown mechanisms.

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17 Hematopoietic Growth Factors in Transfusion Medicine

The availability of hematopoietic growth factors on a large scale for in vivo and in vitro use has opened a new era in transfusion medicine [1–5] (Table 17.1). This chapter focuses on the influence of these growth factors on hematopoiesis in vivo and, thus, the impact of growth factors on transfusion therapy. Chapter 18 describes the use of hematopoietic growth factors in vitro to produce new kinds of blood components for novel therapies.

17.1 Erythropoietin

Description and pharmacology

Erythropoietin (EPO) was the first hematopoietic growth factor identified. The EPO gene is located on chromosome 7. EPO is a glycoprotein with a molecular weight of 34,000 daltons produced primarily in the kidney in adults [6]. EPO behaves like a hormone because it is synthesized in response to hypoxia. EPO stimulates erythroid proliferation and differentiation, and the plasma level of EPO appears to increase at hemoglobin levels of 10.5 g/dL or less [7]. EPO therapy results in an increase in reticulocyte count in about 10 days and increases in hematocrit and hemoglobin in 2–6 weeks. EPO is found widely throughout the evolutionary tree, and therefore EPO produced in many animals is almost identical to human EPO. EPO is being used in several situations (Table 17.2).

Chronic renal failure

EPO was initially used clinically in situations of anemia with low EPO levels. Because EPO is produced in the kidney, it is extremely effective in elevating the hemoglobin and reducing or eliminating the need for transfusion in patients with end-stage renal disease [8, 9]. This treatment has drastically altered the state of these patients, greatly improving their quality of life [10]. EPO is now a standard part of the treatment of patients with chronic renal failure, and it has been estimated that this has eliminated the use of 250,000–500,000 units of red cells annually in the United States [1, 11].

Table 17.1 Some hematopoietic growth factors.

Factor	Major target cell
Erythropoietin	Erythrocyte precursors
G-CSF	Granulocytes, monocytes
GM-CSF	Granulocytes, monocytes, eosinophils, basophils, megakaryocytes, erythrocytes
M-CSF (CSF-1)	Monocytes
IL-3 (Multi-CSF)	Granulocytes, monocytes, eosinophils, basophils, erythrocytes, megakaryocytes, multipotential progenitor cells
c-kit ligand	Synergizes with multiple factors to simulate (stem cell factor) proliferation and differentiation in multiple lineages, pre-B cells
IL-1a and b	Fibroblasts, stem cells
IL-2 (T-cell growth factor)	T cells, activated B cells
IL-4	B and T cells, myeloid cofactor
IL-5	B cells and eosinophils
IL-6	B cells, myeloma cells, megakaryocytes, granulocytes, monocytes, multipotential progenitor cells
IL-7	Pre-B and T lymphocytes, megakaryocytes
IL-9	Helper T cells and erythroid progenitors
IL-11	B cells, megakaryocytes, mast cells
IL-12	Helper T cells, natural killer cells

Source: Cottler-Fox M, Klein HG. Transfusion support of hematology and oncology patients—the role of recombinant hematopoietic growth factors. Arch Pathol Lab Med 1994; 118:417–420.
G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage CSF; IL, interleukin; M-CSF, monocyte CSF.

Anemia of chronic disease

In the anemia of chronic disease, there may be marrow suppression due to the disease itself or the therapy being administered, or there may be decreased synthesis of EPO [12]. In these patients, the EPO levels may be lower than expected from the degree of anemia. These patients use a substantial amount of blood. For instance, it has been estimated that cancer patients use approximately 200,000 units of red cells annually [1]. Thus, strategies to reduce this need would have an important effect on the nation's blood supply. Controlled trials of EPO have shown reductions of 32–85% in blood use have been reported [12]. The American Society of Clinical Oncology and the American Society of Hematology recommend use of EPO for patients with chemotherapy-induced anemia when the hemoglobin is less than 10 g/dL [13]. There is no agreement on the value of EPO when the hemoglobin is between 10 and 12 g/dL and falling although reimbursement is no longer available for EPO use where the hemoglobin is >10 g/dL EPO may be helpful in treating the anemia of multiple myeloma, Hodgkin's disease, myelodysplasia, and chronic lymphocytic leukemia [13,14]. In general, patients with the lowest EPO levels respond the most. EPO therapy has also been shown to elevate the hemoglobin in patients

Table 17.2 Guidelines for EPO therapy.

  I. Currently approved indications
   a. Anemia of chronic renal failure
   b. Anemia in cancer patients undergoing chemotherapy
   c. Reduction of surgical blood loss in anemic patients
   d. Anemia in human immunodeficiency virus infection, in patients undergoing treatment with AZT
 II. Indications under investigation
   a. Anemia of chronic disease, including rheumatoid arthritis and cancer
   b. Donation of blood for autologous use
   c. Bone marrow transplantation
   d. Anemia of prematurity
   e. Myelodysplastic syndromes
III. Current contraindications
   a. Patients with uncontrolled hypertension
   b. Patients with pure red cell aplasia

Sources: Information provided by the Food and Drug Administration. Aher S, Ohlssson A. Cochrane review: Late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Evidence-Based Child Health 2007; 2:426–489. Means RT, Olsen NJ, Krantz SB et al. Treatment of the anemia of rheumatoid arthritis with recombinant human erythropoietin: clinical and in vitro studies. Arthritis & Rheumatism 2005:32; 638–642. Beris P, Soulier-Lauper M, Tullen E, et al. Recombinant human erythropoietin as adjuvant treatment for autologous blood donation: A prospective study. Vox Sang 2009; 62:212—218. Ayash LJ, Elias A, Hunt M, et al. Recombinant human erythropoietin for the treatment of anemia associated with autologous bone marrow transplantation. British J Hematology 2008; 87; 153–161. Jadersten M, Malcovati L, Dybedal I. Erythropoietin and granulocyte-colony stimulating factor treatment associated with improved survival in myelodysplastic syndrome. J Clin Oncology 2008; 26:3607–3613.
EPO, erythropoietin.

with rheumatoid arthritis, but usually these patients do not require transfusions and there is not a concomitant improvement in activity levels or well-being following EPO [14, 15]. The major use of EPO in these patients may be in preparation for elective surgery (see below). Patients with chronic anemia associated with inflammatory bowel disease also experience increases in hemoglobin when receiving EPO [14, 16]. Thus, there are various situations in which the anemia of chronic disease includes low EPO levels or levels inappropriate for the degree of anemia and in which EPO may be of benefit. In patients with chronic anemia other than that due to chemotherapy, the iron stores should be evaluated and, if indicated, iron therapy initiated before resorting to EPO.

Acquired immune deficiency syndrome

Many patients with acquired immune deficiency syndrome (AIDS) are anemic because of the drugs used to treat the HIV infection, marrow suppression associated with the HIV infection, superinfections due to the immunosuppression, and the drugs used to treat the superinfections. In AIDS patients, EPO levels are lower than might be expected for the degree

of anemia present. In patients receiving zidovudine therapy, EPO treatment increases their hemoglobin concentration and reduces the red cell transfusion requirements [17, 18], and it is recommended in AIDS patients who have EPO levels less than 500 mU/mL [14].

Anemia of prematurity

Premature infants are usually anemic because there is diminished EPO production and iatrogenic blood loss due to laboratory testing (see Chapter 12). However, there is some uncertainty whether the response to EPO is normal [19] or impaired [20]. It appears that EPO is helpful in these patients, especially those with a birth weight under 1.3 kg [14, 21, 22].

Autologous blood donors

The use of EPO to stimulate erythropoiesis to increase the amount of blood that autologous donors can provide for their surgery is described in Chapter 6.

Perioperative situations

Some patients planning elective surgery are anemic and thus are not eligible to donate autologous blood. There has been considerable interest in using EPO in the perioperative period to increase the patient's hemoglobin concentration before surgery and to establish more vigorous erythropoiesis at the time of surgery, thus potentially reducing perioperative transfusion requirements. Several studies (reviewed in reference 14) seem to indicate that in general for patients with a hemoglobin less than 13 g/dL, preoperative EPO can reduce the likelihood of transfusion substantially. Iron therapy must also be provided if indicated. As mentioned previously in patients with rheumatoid arthritis, treatment with EPO before surgery results in an increased hemoglobin level at the time of surgery and reduced need for blood in the perioperative period [23].

Bone marrow transplantation

High-dose EPO reduces red cell transfusions following allogeneic bone marrow transplantation [24] but not after blood stem cell or autologous bone marrow transplantation [14], probably because of the relatively rapid engraftment in the latter situations. EPO has been used to stimulate erythropoiesis in allogeneic marrow donors, and in one case this enabled the patient to receive all red cell transfusions from only the marrow donor [25].

Critically Ill patients

Patients in intensive care units (ICU) often receive red cell transfusions due to their underlying condition, blood removed for testing, or marrow hypofunction. Patients in ICUs who receive EPO are less likely to receive transfusions and in one study [26] had a 19% decrease in the number of units of red cells used. EPO treatment of ICU patients has not been shown to improve the clinical outcome and is probably not cost-effective [27].

Patients who refuse blood transfusion

EPO can be used to increase the hemoglobin level in anemic patients who refuse red cell transfusion but who require elective surgery [28] or as a bridge to recovery from anemia in patients who experience acute blood loss but refuse transfusion [29].

Complications of EPO use

With extensive use of EPO and longer-term follow-up of patients, several complications have emerged. Because EPO produced by recombinant DNA methods differs from the native EPO in the carbohydrate portion of the molecule, in rare instances [30, 31] patients receiving chronic EPO therapy have developed antibodies that react with their native EPO causing severe red cell aplasia.

Cardiovascular events such as stroke, thrombosis, and myocardial infarction and more rapid progression of breast, head, and neck and lung cancer have been reported [5, 32, 33].

17.2 Granulocyte colony-stimulating factor

Description and pharmacology

Granulocyte colony-stimulating factor (G-CSF) is a 19,000-dalton protein produced by monocytes, fibroblasts, and endothelial cells [34]. The gene encoding for G-CSF is located on chromosome 17. G-CSF exerts its action by binding to a specific receptor on neutrophil precursors. Following administration of a single dose of G-CSF to humans, plasma levels increase in about 2–8 hours and there is a half clearance time of 3–4 hours [34]. G-CSF increases myeloid production and maturation, thus increasing the size of the myeloid pool, but there is no change in the survival of myeloid cells in the circulation [35, 36]. The granulocyte count begins to increase within a few hours, reaches its maximum at about 12 hours, and gradually returns to baseline by about 2–3 days [34]. The degree of increase in the granulocyte count is related to the dose of G-CSF and the duration of treatment [34, 37]. The in vitro effects of G-CSF include increases in phagocytosis, bactericidal capacity, chemotaxis, and antibody-dependent cell-mediated cytotoxicity [34, 35].

Myeloid growth factors are being used in a variety of clinical situations (Table 17.3).

Neutropenia due to marrow suppression

G-CSF is used in conjunction with chemotherapy in many types of malignancy. Several clinical trials of G-CSF in cancer patients have shown reductions in the period of chemotherapy-induced leukopenia, the number of infectious episodes during neutropenia, the number of febrile episodes, the number of days of treatment with intravenous antibiotics, and the number of days of hospitalization [2, 3, 35, 38, 39]. In patients undergoing either autologous marrow [40–42] or blood stem cell transplants [43, 44], G-CSF decreases the time to neutrophil recovery, days

Table 17.3 Guidelines for myeloid recombinant growth factor therapy.

I	Currently approved indications
	GM-CSF to accelerate myeloid recovery in patients with non-Hodgkin's lymphoma, acute lymphoblastic leukemia, and Hodgkin's disease who are undergoing autologous bone marrow transplantation
	G-CSF to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs that are associated with a significant incidence of severe neutropenia with fever
II	Indications under investigation
	To accelerate myeloid recovery in patients with myelodysplastic syndrome, AIDS, marrow graft failure, peripheral blood stem cells (PBSCs) transplantation, congenital agranulocytosis, or malignancies not mentioned above
III	Current contraindications
	GM-CSF in patients with excessive leukemia myeloid blasts in the bone marrow or peripheral blood (>10%); known hypersensitivity to GM-CSF, yeast-derived products, or any component of the product
	G-CSF in patients with known hypersensitivity to *Escherichia coli*-derived proteins

Source: Goodnough LT, Anderson KC, Kurtz S, et al. Indications and guidelines for the use of hematopoietic growth factors. Transfusion 1993; 33:944–959. G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor.

of fever or antibiotic use, and length of hospital stay. Allogeneic marrow or blood stem cell transplant patients receiving G-CSF have a shorter time to neutrophil engraftment and length of stay but no difference in other clinical parameters [3, 35, 41, 45–47].

Although the use of G-CSF in these marrow suppression situations shortens the period of neutropenia, it probably will not greatly alter transfusion therapy in the near future, since leukocyte replacement is not widely practiced. However, reducing the incidence or the severity of infection could modify transfusion therapy if sepsis and other complications are avoided, with resulting decline in the use of platelets and fresh frozen plasma.

Chronic benign neutropenia

G-CSF increases the granulocyte count in patients with severe chronic benign agranulocytosis [35, 48]. This is associated with decreases in preexisting infections, new infections, and use of intravenous antibiotics. The long-term effects of G-CSF treatment in these patients is a subject of great interest because of the concern that long-term therapy with growth factors might increase the likelihood of the development of myeloid malignancy [5, 49].

AIDS

AIDS patients may be neutropenic because of the disease, the chemotherapeutic agents, or infections and the chronic disease state. A few studies have suggested that G-CSF may be beneficial in the neutropenia of AIDS [35], although G-CSF is not used routinely.

Aplastic anemia

The overall value of G-CSF in aplastic anemia is not established. G-CSF has been administered to a few patients with aplastic anemia with mixed results [2, 35]. Some patients experienced an increase in granulocyte count, while others did not. This is not surprising, since aplastic anemia is a disease of marrow failure, and there is often little response to administration of growth factors [35].

Stem cell mobilization in normal donors

Hematopoietic stem cells circulate in the peripheral blood and can be collected using standard apheresis procedures (see Chapter 7). The number of stem cells usually circulating is very small but increases as a rebound after chemotherapy. This situation was used to advantage, and the techniques for collection and initial experiences with the collection and transfusion/transplantation of peripheral blood stem cells (PBSCs) were developed in autologous donor-patients. A few studies also included administration of G-CSF to further increase the number of circulating stem cells [35, 50, 51]. The number of PBSCs that can be obtained from normal donors is not adequate for transplantation. However, G-CSF can be used to increase the level of circulating stem cells making it possible to accomplish allogeneic transplantation [52–57].

Thus, it is important to understand the effects of G-CSF on normal donors [34, 35, 37]. In normal donors, G-CSF causes an increase in circulating CD34+ cells beginning at about 3 days and reaching a peak after 5–6 days (Figure 17.1). The increase is dose dependent, and the levels of CD34+ cells begin to decline by about day 8, even if G-CSF is continued (Figure 17.2). With the levels of circulating CD34+ cells achieved, a
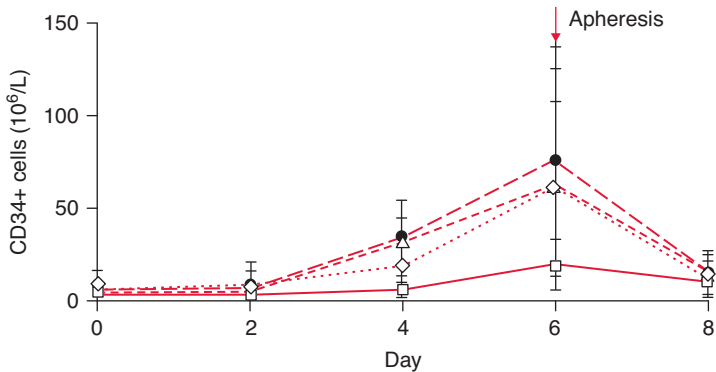
Figure 17.1 The number of CD34+ cells in donors given 5 days of granulocyte colony-stimulating factor (G-CSF). The subjects were given 2 (——□——) (n = 5), 5 (··· ◇ ···) (n = 16), 7.5 (— △ – –) (n = 27), or 10 (—●—) (n = 21) mg/kg per day, and peripheral blood stem cells (PBSCs) were collected on day 6. (Reproduced with permission from Stroncek DF, Clay ME, Petzoldt ML, et al. Treatment of normal individuals with granulocyte-colony-stimulating factor: donor experiences and the effects on peripheral blood CD34+ cell counts and on the collection of peripheral blood stem cells. Transfusion 1996; 36:601–610.)
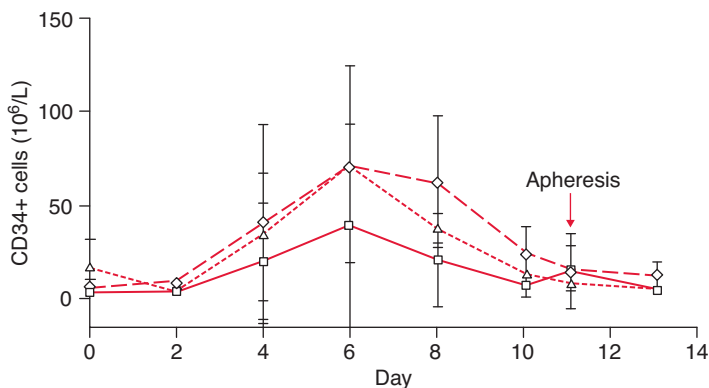
Figure 17.2 The number of CD34+ cells in donors given 10 days of granulocyte colony-stimulating factor (G-CSF). The subjects were given 2 (—— □ ——) ($n = 6$), 5 (– – ◇ – –) ($n = 7$), or 7.5 (··· △ ···) ($n = 2$) mg/kg per day, and peripheral blood stem cells (PBSCs) were collected on day 11. (Reproduced with permission from Stroncek DF, Clay ME, Petzoldt ML, et al. Treatment of normal individuals with granulocyte-colony-stimulating factor: donor experiences and the effects on peripheral blood CD34+ cell counts and on the collection of peripheral blood stem cells. Transfusion 1996; 36:601–610.)

cytapheresis procedure processing about 9 L of whole blood produces approximately 30×10^9 mononuclear cells and 400×10^6 CD34+ cells [54, 55]. Approximately 50% of cytapheresis procedures yield a dose of CD34+ cells adequate to transplant a 75-kg recipient, and two procedures provide an adequate cell dose in 90% of cases [54]. The dose of CD34+ cells can be increased by processing larger volumes of blood, and the major limitation of this is the donor's ability to tolerate being immobilized by the blood cell separator. Present donation procedures attempt to process about 15 L of blood within about 5 hours [55], which yields in a single procedure a dose of cells adequate to transplant most adults [55]. The procedures for stem cell collection and the composition of the stem cell concentrate are described in detail in Chapter 7.

The next issue is the safety of this procedure in normal donors. Fortunately, a considerable amount of information is available to answer this issue. Most normal donors who receive G-CSF experience side effects [37, 56]. The most common are headache, myalgia, bone pain, and flu-like symptoms (Table 17.4). These side effects are more severe in females and in donors receiving higher doses of G-CSF [37]. There are also some biochemical and electrolyte changes (Table 17.5), but these are not clinically significant. The changes include increased alkaline phosphatase, ALT, LDH, and sodium and decreased glucose, potassium, bilirubin, and BUN [37]. Following cytapheresis for the collection of stem cells, there is a 36% decrease in platelet count and a rebound to levels above baseline at about 2 weeks [37], followed by return to baseline by 1 month [58] (Figure 17.3). The decrease in platelet count is greater than can be accounted for by the number of platelets collected in the stem cell concentrate [37], suggesting an effect of G-CSF on megakaryopoiesis. The

Table 17.4 Experiences of people given G-CSF for 5 or 10 days.

	All donors	
	N	%
Bone pain	85	83
Headache	40	39
Body aches	23	23
Fatigue	14	14
Nausea and/or vomiting	12	12
Redness, swelling, or warmth at the injection site	7	7
Feeling hot and/or having night sweats	7	7
Trouble sleeping	4	4
Skin pain and/or itching	2	2
Enlarged and/or painful lymph nodes	—	—
Arthralgias	2	2
Shortness of breath	2	2
Irritability	1	1
Feeling dazed	1	1
Feeling foggy-headed	1	1
Skin rash	1	1
Took acetaminophen or ibuprofen	1	1

Source: Stroncek DF, Clay ME, Petzoldt ML, et al. Treatment of normal individuals with granulocyte-colony-stimulating factor: donor experiences and the effects on peripheral blood CD34+ cell counts and on the collection of peripheral blood stem cells. Transfusion 1996; 36:601–610.

Table 17.5 Serum chemistries in people given G-CSF for 5 days.[a]

Test	Day 6
Sodium (mmol/L)	142 ± 2[b]
Potassium (mmol/L)	37 ± 0.4[b]
Chloride (mmol/L)	102 ± 3
Bicarbonate (mmol/L)	27 ± 3
BUN (mg/dL)	10 ± 3
Creatinine (mg/dL)	1.0 ± 0.2
Glucose (mg/dL)	103 ± 12
Alkaline phosphatase (U/L)	179 ± 43[b]
ALT (U/L)	38 ± 21[c]
Bilirubin (mg/dL)	0.4 ± 0.2[b]
Calcium (mg/dL)	9.4 ± 0.4
g-Glutamyl transferase (U/L)31 ± 17	31 ± 17
LDH (U/L)	1307 ± 363[b]

Source: Stroncek DF, Clay ME, Petzoldt ML, et al. Treatment of normal individuals with granulocyte-colony-stimulating factor: donor experiences and the effects on peripheral blood CD34+ cell counts and on the collection of peripheral blood stem cells. Transfusion 1996; 36:601–610.

G-CSF, granulocyte colony-stimulating factor.

[a]Values represent the mean $\pm$ 1 SD.

[b]Compared with day 0, $p < .01$.

[c]Compared with day 0, $p < .05$.
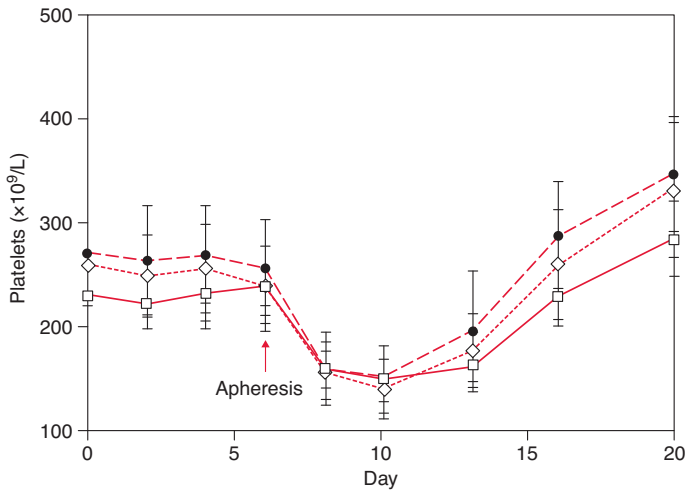
Figure 17.3 Peripheral blood platelet counts in healthy people who were given granulocyte colony-stimulating factor (G-CSF) for 5 days (days 1–5) and donated peripheral blood stem cells (PBSCs) (day 6, arrow). Donors were given 5 (——— □ ———) ($n = 9$), 7.5 (– – ● – –) ($n = 31$), or 10 (··· ◇ ···) ($n = 20$) mg/kg per day of G-CSF. Values represent mean 61 SD. (Reproduced with permission from Stroncek DF, Clay ME, Smith J, Ilstrup S, Oldham F, McCullough J. Changes in blood counts after the administration of peripheral blood stem cells from healthy donors. Transfusion 1996; 36:596–600.)

neutrophil levels, which are elevated at the time of cytapheresis, decrease immediately but do not return to baseline until about 2 weeks [37].

The long-term effects of G-CSF on normal donors are still being studied. The major concern is whether the stimulation of hematopoiesis by G-CSF might lead to hematopoietic malignancy in individuals somehow predisposed to this. One case of hematopoietic malignancy has occurred in a patient receiving long-term G-CSF [49], but at present there is no indication that a short course of G-CSF as is given to PBSC donors poses a long-term risk to normal donors. Blood counts in normal donors are unchanged from baseline 12 months after an initial exposure to G-CSF and PBSC donation [58], and the PBSC yield from a second donation 1 year later is similar to the original donation [58]. Thus, these initial follow-up studies do not indicate any long-term adverse effects of G-CSF [5, 59].

G-CSF-mobilized PBSCs collected from normal donors has assumed an important role in hematopoietic transplantation [60]. This is the second major use of a strategy to stimulate donors to produce a blood component. The stimulation of normal donors was used originally when corticosteroids were administered to granulocyte donors. The availability of hematopoietic growth factors may launch a new era in donor stimulation and a new, larger-scale strategy for the mobilization of cells for new therapeutic uses.

Granulocyte mobilization in normal donors

G-CSF has been used to increase the level of circulating granulocytes in normal donors in order to increase the yield of granulocytes collected for

transfusion. Bensinger et al. [61] were able to increase the donor's granulocyte levels by an average of tenfold to 29,600, which resulted in collection of approximately 4×10^{10} granulocytes for transfusion. In another study, G-CSF alone increased the granulocyte count to about 25,000 at 12 hours, but when combined with dexa-methasone the granulocyte count increased to 35,000 (300 mg of G-CSF) or 45,000 (600 mg of G-CSF) [62]. The use of G-CSF in these situations resulting in a much larger dose of granulocytes has led to renewed interest in granulocyte transfusions (see Chapter 11).

17.3 Thrombopoietin

Description and pharmacology

Platelet transfusions have increased more rapidly than those of other blood components and currently constitute a very large part of the activity of most large blood banks. The ability to shorten the duration of thrombocytopenia, reduce the risk of hemorrhage, and reduce the need for platelet transfusion would have great potential benefit.

There was an interesting pathway [63] to identification of thrombopoietin (TPO). The murine myeloproliferative leukemia virus was shown to cause a proliferation of hematopoietic progenitors with cell lines of multiple hematopoietic lineages arising. This mpl ligand was found to increase megakaryocyte size and ploidy and behave as TPO was expected [64–67]. The mpl receptor is found on CD34+ cells, megakaryocytes, and platelets, and mpl ligand (TPO) promotes cell division and the differentiation of cells into megakaryocytes and platelets.

TPO is a 38,000-dalton protein that is glycosylated to form a 90,000-dalton active protein [63, 68] with two domains, one with erythropoietic-like activity that provides the thrombopoietic activity, but with a very short in vivo this domain half-life [63, 68]. Two different TPO agents were developed: one the full TPO molecule and the other the EPO-like domain coupled with polyethylene glycol to provide stability in vivo. The former agent is called TPO, and the latter was called PEG megakaryocyte growth and development factor (MGDF). The site of production of thrombopoietic activity is the liver and kidneys, with minor amounts produced in the bone marrow, spleen, testes, muscle, and brain. Production of TPO is relatively fixed and levels of circulating TPO are determined by platelets (and possibly megakaryocytes) absorbing circulating TPO. Thus, at lower platelet counts, less TPO is absorbed and circulating TPO increases stimulating platelet production. TPO and MGDF cause an increase in the number, size, and ploidy of megakaryocytes, speed the maturation of megakaryocytes into platelets [63] with normal function in vitro [63, 68, 69].

Thrombocytopenia due to marrow suppression

In patients with thrombocytopenia due to chemotherapy for advanced cancer, administration of MGDF increases the platelet count beginning at

about day 6 and peaking at about day 12 [70]. The degree of thrombocytopenia is reduced and the period of thrombocytopenia shorted. Although TPO can decrease platelet utilization after chemotherapy [71–73], this is a clinical situation in which thrombocytopenia is not severe and patients do not require many platelet transfusions. In patients with myeloablation, such as those undergoing hematopoietic stem cell transplantation, TPO has not shortened the time to platelet recovery or decreased the use of platelet transfusions [73–76]. Thus, the exciting potential for TPO has not been realized because it is not effective in the setting in which most bleeding and platelet transfusion occurs.

Stimulation of megakaryopoiesis in normal platelet donors

In some preliminary studies, MGDF was administered to normal plateletpheresis donors to increase the level of circulating platelets and thus the yield of platelets [77]. Transfusion of these high-dose platelet products resulted in huge increase in platelet count suggesting that MGDF could be used to enable the collection of two to four times more platelets than are currently obtained [77, 78]. Unfortunately, MGDF stimulated formation of antibodies that cross-reacted with native TPO, resulting in thrombocytopenia in other normal research subjects [79]. Thus, this potential use of TPO has not developed. Presently TPO is not widely used and has had little impact in transfusion medicine.

17.4 Second generation thrombopoietic growth factors

These are TPO peptide or nonpeptide memetrics or TPO agonist antibodies [80]. The peptide memetrics activate the TPO receptor but the peptide and nonpeptide have different mechanisms of action. TPO agonist antibodies are monoclonals that bind the TPO receptor. The peptide and nonpeptide (eltrombopag) have both increased the platelet counts of ITP patients [81–85] and in patients with hepatitis C [86] for rather long periods with no serious side effects. The effect of these new agents in chemotherapy-induced thrombocytopenia has not been established.

17.5 In vitro uses of hematopoietic growth factors in transfusion medicine

In addition to the use of hematopoietic growth factors in vivo as described in this chapter, many growth factors are being used in vitro to stimulate the proliferation or maturation of hematopoietic cells in culture systems to produce larger numbers of the desired cells. Examples of these in vitro uses of growth factors are expansion of myeloid cells, expansion of CD34 (stem) cells, generation of lymphokine-activated killer cells or activated natural killer cells, production of cells with specific cytotoxicity against

Epstein–Barr virus or cytomegalovirus, or expansion of cells during transduction for gene therapy. These activities are described more completely in Chapter 18.

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18 Cellular Engineering for the Production of New Blood Components

18.1 Present generations of blood components

Developments in the understanding of hematopoiesis, the identification of hematopoietic growth factors and cytokines, and advances in technology for cell separation and culture are being combined to create innovative ways to manipulate blood cells in the laboratory to create new kinds of blood components for new therapeutic uses [1]. In addition, hematopoietic growth factors can be used to stimulate normal donors for the collection of new blood components (see Chapters 7 and 17). These new components involve the use of hematopoietic cells for transplantation, immunotherapy, antimicrobial therapy, or gene therapy (Table 18.1).

18.2 Development and production of new blood components

The development of new components to provide novel cellular therapies involves four general activities: (a) basic research involving the development of novel culture techniques, cell isolation techniques, growth factors, cytokines, monoclonal antibodies, or improved understanding of cell growth characteristics; (b) translational research involving methods development, scaling up the research procedures so they can be carried out on an operational basis with proper safety, cell handling, quality control, documentation, and conformance with regulatory requirements; (c) production of the new components using state-of-the-art technology and quality systems; and (d) clinical trials and patient therapy, in which the "production" versions of the research developments are used on a scale that makes possible decisions about their overall value in patient care. The basic and translational research processes are well understood. The production activity is complex and requires conformance to standard operating procedures, quality control, and conduct under an investigational new drug (IND) authorization [2–4] (Table 18.2). The clinical trials activity is well known and involves the use of these new therapeutic agents on a scale that makes possible decisions about their role in medical care.

Table 18.1 Clinical uses of engineered cells.

Cancer
    Direct toxicity (e.g., NK)
    Indirect via hematopoietic transplant
    Indirect via adoptive immunotherapy
Transplant
    Facilitate engraftment
    Decrease GVHD
    Speed engraftment
Anti-microbial/infectious diseases
    Indirect via immunologic
    Indirect via cytotoxic T cells
    Indirect by speeding hematopoietic engraftment
Regenerative medicine
    Replacing damaged tissue
    Providing tissue-specific progenitor cells
    Providing growth factors, edelivery system
Ex vivo production of blood cells for transfusion
Tissue engineering
    Heart valves
    Blood vessels
    Pancreatic islet cells
    Cartilages
    Cornea
    Skin
Immune diseases
    T regulatory cells
Cardiovascular diseases
    Progenitor cells for repair
Gene therapy

The skills and facilities needed for each of these four activities in the development of a new cell therapy are different. A close working relationship and the integration of these activities are essential for a potential new cell therapy to move effectively from a concept through basic research into production and through clinical trials. The basic research and clinical trials activities are familiar; however, the complexity of the method scale-up and production activity is not well appreciated. The development of new techniques for isolating certain cell populations or manipulating the cells in vitro to produce the novel cell preparation is carried out in research laboratories, usually with small numbers of cells or small volumes of a cell suspension. These research techniques must then be scaled up and adapted into a practical operational method to produce cells for human use. This begins with procedures to collect the large numbers of cells needed for the final product. The cells of interest may need to be isolated from the heterogeneous starting mixture, but the scale of this selection step may pose substantial difficulties. The cells may then be activated or expanded using culture techniques in vitro and any of these various cell suspensions may require preservation for short or long periods. All of these

Table 18.2 Activities involved in the production of new blood components by cellular engineering.

Quality control and quality assurance
Complete documentation of all steps of procedure, reagents used, staff qualifications, equipment validation, and process validation
Interaction with manufacturers of reagents and equipment to obtain proper licensure and approval for use
Interaction with the US Food and Drug Administration (FDA)
Development of appropriate manufacturing information for investigational new drug (IND) submission
Producing cells using producers that comply with FDA good manufacturing practices (GMPs)
Conducting activities in a facility that meets the FDA expectations for good laboratory practices and good manufacturing practices
Conducting a regulatory affairs program that meets the FDA expectations
Expertise in handling blood components
Expertise in the separation of blood into its different cellular components
Preservation of blood cells
Safety programs for laboratory personnel
Development or application of new technology such as special containers, cell separators, cell harvesters, culture systems, etc.
Data handling
Specimen identification

methods must result in a cell suspension with the desired properties but accomplished efficiently, at minimum cost, maintaining safety of the cell suspension and laboratory personnel, and in a manner, enabling the treatment of many patients simultaneously. Almost always, the original research technique must be modified in some way because it does not lend itself to a "production" technology.

18.3 Hematopoietic progenitor and stem cells as a blood component

Increasing variety of sources of stem cells

The variety of these new components is impressive because of the increase in the number and variety of sources of cells and the increasing types of processing procedures [2–6]. For many years, almost the only source of cells was marrow and this was usually from a relative of the patient. Beginning in the mid-1980s, with the use of unrelated marrow donors, the variety began to increase. The current sources of hematopoietic stem cells include bone marrow, peripheral blood, and umbilical cord blood (UCB). These may be obtained from matched siblings, partially matched relatives, the patient himself or herself, unrelated volunteers, and, most recently, UCB from unrelated volunteer mothers (Table 18.3).

These hematopoietic progenitor cells are subjected to an increased variety of processes or manipulations (Table 18.4). Many of these

Table 18.3 Increasing variety of hematopoietic stem cells used as source material.

Marrow
Sibling
Patient
Partially matched relative
Unrelated volunteer
Peripheral blood stem cells
Patient
Sibling
Partially matched relative
Unrelated volunteer
Umbilical cord blood
Sibling
Unrelated volunteer

procedures are performed on either marrow, peripheral blood, or cord blood. This combination of cell sources and manipulative procedures has led to a greatly increased variety of final products being used for transplantation [2, 3].

Hematopoietic stem cell collection
Marrow
Bone marrow is collected by needle puncture of the posterior iliac crests. Usually one operator works on each side while the donor is under either general or spinal anesthetic [7–9]. The marrow is placed into plastic bags through an injection port. Cell counts are done periodically during marrow collection to obtain a total nucleated cell dose of $2–5 \times 10^6$/kg recipient body weight. This results in a marrow component that is then subjected to red cell or plasma depletion, cryopreservation, or other cellular engineering procedures. Marrow donation is a low-risk procedure but fatalities have occurred [8, 9], and there is moderate discomfort for several days after [10]. The development of the US National Marrow Donor Program led to considerable attention to donor selection and management to minimize the risks [10, 11]. The size of the recipient can be used to estimate the volume of marrow that will be collected, which can be used to predict the need for red cell replacement [12]. These red cell needs can be managed by preoperative autologous donation [12] in order to avoid exposing the marrow donor to allogeneic red cells. The use of marrow for transplant has decreased and is replaced by peripheral blood stem cells (PBSCs) [7].

Peripheral blood stem cells
Collection of PBSCs is described in Chapter 7.

Umbilical cord blood
Collection and banking are described later in this chapter.

Table 18.4 Examples of process, cells, and products involved in novel cellular therapies.

Progenitor cells
Cardiac progenitor cells
Corneal progenitor cells
Hematopoietic progenitor cells (HPC)
    Peripheral blood (fresh and cryopreserved)
    Bone marrow (fresh and cryopreserved)
    Bone marrow mononuclear cells (unmanipulated)
    Bone marrow mononuclear cells (CD34 selected)
    Umbilical placental/cord blood—ex vivo expansion
Hepatitic progenitor cells
Human embryonic stems cells (HESC)
Induced pluripotent stem cells (IPS)
Neural progenitor cells
Mesenchymal stem cells (adipose tissue, lung, marrow, amniotic, Wharton's jelly)
Skeletal myoblasts

Cell depletion/Cell enrichment
Bone marrow/peripheral blood/UCB
    CD3 depletion
    CD34 selection
    CD133 selection
    CD34+/CD3
    CD56 selection
    Counterflow elutriation

Dendritic cells
Adenovirally transduced
Apoptotic tumor cell pulsed
Peptide pulsed
Transfected
Tumor lysate pulsed
Tumor-dendritic cell hybrids

Donor leukocytes
Donor leukocyte infusion (DLI)
Alloreactive T cell depleted (immunotoxin)
Thymidine kinase (suicide gene)-transduced T cells

Genetically modified cells
Activated T cells
Fibroblasts
Cytotoxic T-lymphocytes (CTLs)
Hematopoietic stem cells (HSC)
Lymphoblastoid cell lines (LCLs)
Mesenchymal stem cells (plasmid or viral vector)
Neural stem cells
Tumor cells
Tumor vaccines (autologous, allogeneic)

Antigen presenting cells
Dendritic cells
Leukemic cell lines
Monocytes

Table 18.4 (*Continued*).

Lymphocytes
Peripheral blood-derived lymphocytes
   Lymphocyte activated killer (LAK) cells
   Activated natural killer (NK) cells
   Invariant NK T cells
   CD8+/CD4+ T cells
   CD4+/CD25+ T regulatory cells
Umbilical cord blood-derived lymphocytes
   CD4+/CD25+ T regulatory cells
Cytotoxic T-lymphocytes (CTLs)
   EBV-directed CTLs
   Adenovirus-directed CTLs
   EBV-LMP-directed CTLs
   Tri-virus-directed CTLs (Adenovirus/CMV/EBV)
   Genetically modified CTLs (TGFβ, chimeric antigen receptors)
EBV-transformed B cell lines (LCLs)
LCLs +/− genetic modification—intermediate product

Master cell bank/working cell bank
Artificial antigen presenting cells (K562)
Fibroblasts
Glioblastoma
Human embryonic stem cells
Mesenchymal stem cells
Neuroblastoma
NK cells lines

Tumor vaccines (translational development)
CLL-directed vaccine (autologous)
Large multivalent immuogen (LMI) vaccine (autologous)
   Breast adenocarcinoma
   Melanoma
   Renal cell carcinoma
Neuroblastoma-directed vaccine (autologous, allogeneic)

Other
Aseptic filling
B95-8 EBV
Culture and expansion of cells from normal tissue
Cell expansion
Immune monitoring
Large animal models
Potency assay development
Cryopreservation technologies
Monoclonal antibodies
Plasmids
Recombinant proteins
Suspension and adherent cell banks

Hematopoietic stem cell preservation
Liquid preservation

In unrelated donor marrow or blood stem cell transplants, the donor may be in a distant location from the patient, and in cord blood banking, the blood may be collected in a location distant from the processing laboratory. Therefore, methods for the short-term liquid storage of hematopoietic stem cells are important. Initial work demonstrated that tissue culture solutions can successfully maintain marrow, blood, and cord blood stem cells (CBSCs) at room temperature or 4°C for 24 hours [13–16]. However, tissue culture solutions are not designed for in vivo infusion. Some solutions suitable for infusion such as Plasmalyte A (Baxter Healthcare) and Normosol (Abbott Laboratories) also maintain marrow, blood, and CBSCs for at least 24 hours [17–19]. A solution, STM-Sav, designed for hematopoietic stem cell liquid preservation is equally effective [17–19], but none of these three solutions is licensed by the FDA for stem cell preservation. It is clear that adequate recovery of mononuclear cells, CD34+ cells, and CFU-GM colonies can be obtained after storage of hematopoietic stem cells for 24 hours at room temperature or 4°C [17–19]. Some centers prefer to store cells at room temperature, while others prefer 4°C to minimize bacterial growth in case contamination occurred during collection. Short-term liquid storage of hematopoietic stem cell products remains a local decision, as standards do not yet specify the minimum or optimum conditions.

Cryopreservation

Many cell processing methods lead to a cell suspension of varying composition for which frozen preservation is desired. Despite the variety of manipulations of the source or starting material, the freezing and storage methods themselves were developed years ago and are rather standard [20, 21]. The usual method for freezing stem cells uses liquid nitrogen as the freezing system, dimethyl sulfoxide (DMSO) as the cryoprotectant, and controlled-rate freezing. The most critical stages in the process are freezing and thawing. If the cooling rate is too slow, extracellular ice crystals form and damage the cells due to the increased osmolarity of the external solution. If the cooling rate is too fast, intracellular ice crystals form and damage the cells, leading to lysis. The cryoprotective agent, DMSO, binds to water and slows the formation of ice crystals both internally and externally, thus protecting the cell. A particularly important stage in the freezing process is the transition from liquid to solid. The heat of fusion is released then, and the time required to pass through this phase is important. A programmed freezing apparatus is used to control the entire freezing process [20]. The best cooling rate is −1°C to −3°C per minute [20–22]. When freezing is complete, the cells are stored in liquid nitrogen at about −196°C. This provides better preservation than higher temperatures [21, 23], and these conditions preserve stem cells for up to 11 years [24].

There are toxicities associated with marrow transfusion, and these seem to be related to the amount of DMSO the patient receives [25]. In an effort

to minimize the amount of DMSO administered and also to simplify the freezing procedure, a combination of lower concentrations of DMSO, along with hydroxyethyl starch and albumin, have been used [26–29]. In this system, the stem cells suspended in the cryoprotectant are placed in special containers and frozen in a mechanical freezer at about $-80°C$ that provides freezing rates about the same as in the controlled-rate liquid nitrogen system [26, 28, 29], and the cells are protected satisfactorily [26–29]. The cells are also stored at $-80°C$ in a mechanical freezer, reducing the cost and complexity of the system. The maximum duration of storage in this system is not established but is at least five years [29, 30].

Thawing is another important step in the use of hematopoietic stem cells. Except for UCB, hematopoietic stem cells are usually thawed at the bedside to minimize their contact with DMSO [31, 32]. This is discussed along with infusion of hematopoietic stem cells (see Chapter 13).

Hematopoietic stem cell products
Bone marrow
The characteristics of marrow as collected for transplantation depend on the size of the recipient and the technique for marrow aspiration, which determines the amount of blood that dilutes the marrow cells. Because marrow is collected to obtain a nucleated cell dose of $2–5 \times 10^6$/kg recipient body weight, the volume of marrow may range from about 200 mL to 1500 mL or even more. When smaller amounts of marrow are aspirated from each puncture site, the hematocrit will be lower compared with hematocrits up to 25% or even 30% if aspirations from a single site draw blood. Thus, it is difficult to define a "standard" marrow product. An approximation for comparative purposes is shown in Table 18.5.

Peripheral blood stem cells
Hematopoietic progenitor cells circulate in the peripheral blood, although in relatively smaller numbers than in the bone marrow. Following

Table 18.5 Approximate composition of hematopoietic stem cell products after collection from the donor and before cellular engineering.

	Marrow	PBSC	UCB
Volume (mL)	870	250	109
Hematocrit (%)	53	4	33
RBC mass (mL)	461	10	36
Total nucleated cells ($\times 10^{10}$)	1.5	1.7	1.2
Mononuclear cells ($\times 10^9$)	3.2	30	0.5
CD34+ cells ($\times 10^8$)	1.39	4	NA
Platelets ($\times 10^{11}$)	NA	4	NA

NA, not available; PBSC, peripheral blood stem cells; UCB, umbilical cord blood.

chemotherapy, there is a rebound phenomenon and large numbers of PBSCs can be collected for autologous transplantation. In normal allogeneic donors, the number of circulating stem cells is inadequate to obtain enough cells for transplantation. The level of circulating PBSCs can be increased by administering the growth factor granulocyte colony-stimulating factor (G-CSF) (see Chapter 17). In normal subjects, the administration of G-CSF causes an increase in the percentage of CD34+ cells from 0.05% before treatment to about 1.5% after 5 days [33–36]. This results in a yield of about 4.5×10^8 CD34+ cells from a single apheresis [33, 34]. The usual dose of CD34+ cells considered suitable for transplantation is about $2.5–5 \times 10^6$/kg or about 2×10^8 for a 70-kg person. Thus, one such apheresis concentrate is usually adequate for a transplant. The side effects of G-CSF administration in normal donors include bone pain, headache, and flu-like symptoms but are manageable with analgesics [33]. PBSCs are being used increasingly for allogeneic transplantation [35–40], thus eliminating marrow collection in the operating suite, along with the attendant risks of anesthesia and the marrow collection process.

Umbilical cord blood

Bone marrow transplantation (BMT) is an effective and successful form of therapy that is being used to treat an increasing number of diseases in an increasing number of institutions. Matching a donor and recipient is so complex that for many years transplantation was considered feasible only between HLA-matched siblings. A few patients without HLA-matched siblings could receive an autologous transplant or one from a partially HLA-matched relative. However, donors still were available for only about 40% of patients [41] until it was established that transplantation can be successful using marrow from properly matched unrelated individuals [42, 43]. However, graft failure and graft-versus-host disease (GVHD) remain substantial issues in successful BMT. Therefore, there has been considerable interest in alternative sources of marrow cells that might reduce or eliminate these problems and increase the availability of transplantation for patients who lack HLA-identical siblings.

Pluripotent hematopoietic stem cells are abundant in human UCB [44, 45]. Successful hematopoietic reconstitution has been accomplished using CBSCs [46–54]. Initially, these transplants were done using HLA-matched sibling donors; however, a large number have also been done using blood from donors with two or three HLA antigens mismatched with the recipient [55] with apparently less GVHD and less stringent HLA matching requirements [55]. Thus, cord blood has these potential advantages over marrow and also is free of contamination with latent viruses (cytomegalovirus, Epstein–Barr virus) and a low number of GVHD-producing T lymphocytes [44, 45]. As cord blood transplants have increased, cord blood banks have been developed. Cord blood banking is described later in this chapter.

18.4 General hematopoietic cellular engineering processes

Cell depletion
Red cells
The first marrow cellular manipulation procedure was the removal of plasma when the patient was too small to tolerate the volume of marrow. Soon thereafter, procedures were developed for the depletion of red cells to avoid hemolysis when the marrow was ABO incompatible with the recipient (see Chapter 12) [56–60]. Initially this was done in plastic bags using sedimenting agents such as hydroxyethyl starch and ordinary blood component handling techniques. However, because of the nature of the component being manipulated, the procedure seemed far from ordinary. This approach may be used not only for ABO incompatibility but also for any clinically significant red cell incompatibility in which the patient has a red cell antibody and the donor is positive for the corresponding antigen.

T lymphocytes
One of the severe complications of marrow transplantation is GVHD caused by donor lymphocytes. A major strategy to reduce GVHD was the depletion of marrow or PBSC T lymphocytes [61] by T-cell rosetting, monoclonal antibodies plus complement [62], immunotoxins [63], and physical separation by elutriation [64]. The general experience with these methods was that T-cell depletion seemed to reduce GVHD but was also associated with a higher rate of graft failure. Thus, T-depletion has not been adopted as a routine process.

Tumor cell purging
For a while, processes were used to remove tumor cells from marrow being used for autologous transplantation in patients with solid tumors or hematologic malignancies [65]. The marrow was collected when the patient was in remission and there was thought to be little or no marrow involvement. Methods used for depleting residual tumor cells include monoclonal antibodies plus complement or immunotoxins, immunomagnetic bead separation using monoclonal antibodies, or cytotoxicity using pharmacologic agents such as 4-hydroxy-cyclophosphamide [65–67]. This approach did not prove to be clinically effective.

Positive selection
The methods to positively select stem cells from marrow or peripheral blood involve selection of CD34+ cells [68]. The CliniMACS (Miltenyi Biotec) uses an anti-CD34 monoclonal antibody complexed to iron-dextran magnetic particles to select CD34+ cells by passing the suspension through a magnetic field. The CD34 selection steps are carried out as part of many different processes such as isolation of CD34+ cells

from peripheral blood for transplantation or as starting material for subsequent ex vivo expansion or as part of T-cell depletion protocols. Positive selection of CD34+ cells has become the first step in preparing many of the hovel cell products.

Expansion

The term expansion has become jargon but is really incorrect. Individual cells are not increased in size. The term refers to culture systems that increase the number of cells.

Hematopoietic growth factors can be used in ex vivo culture systems to increase the number of cells and thus produce a component in the laboratory that is not obtainable directly from a donor. The major interest has been to increase the number of hematopoietic stem cells so that transplants could be done after obtaining smaller numbers of cells from donors. There is particular interest in expanding UCB stem cells because of the small number usually collected. None of these efforts has been successful. A 20- to 60-fold increase in myeloid progenitors can be achieved after about 14 days of culture with growth factors [69, 70]. These cells might be used as an adjunct in BMT to shorten the period of neutropenia [71]. While this might lead to a reduction in infections and related morbidity, this practice has not been widely adopted.

Lymphocyte expansion is described with adoptive immunotherapy.

Activation

Cytokines and interleukins can be used to activate mononuclear cells usually to achieve antitumor effects. This is described in the section on adoptive immunotherapy.

Transduction

Hematopoietic cells, either lymphocytes or CD34+ cells, can be genetically modified and used in gene therapy. This is described in the section on gene therapy.

Types of cells used in cellular engineering
Lymphoid cells

It has been possible for years to maintain or expand lymphocytes in culture. The availability of hematopoietic growth factors and new devices or containers has improved these culture systems. The lymphocyte culture/expansion procedures are done to produce large numbers of cells for immunotherapy. Because of the variety and extent of these activities, they are described under adoptive immunotherapy below. Lymphocyte expansion is also carried out as part of gene therapy, and those activities are described below under gene therapy.

Megakaryocytes and platelets

Just as G-CSF and other growth factor combinations can be used to increase the number of myeloid cells in vitro, thrombopoietin (TPO) has been used in in vitro culture systems to increase the number of

megakaryocyte progenitors by 7–14 times and eliminate the need for platelet transfusions in some patients [72, 73]. Because TPO has a stimulatory effect on early progenitors, the clinical effectiveness of cells generated by the use of TPO in these ex vivo systems is not yet established.

18.5 Umbilical cord blood banking

As cord blood has become a major source of cells for hematopoietic transplantation, it was necessary to establish "banks" of stored UCB. Many issues that must be resolved, specific procedures be developed and requirements to be defined as these banks have been established (Table 18.6) [74, 75]. Many blood banks have become involved in cord blood banking because it draws upon the strengths and experiences of traditional blood banking, along with more recent experiences with marrow and cord blood transplantation [1, 75, 76].

Obtaining consent

Written consent must be obtained from the mother authorizing placement of the cord blood in the bank and using the blood for transplantation. Preferably the consent would be obtained during prenatal care prior to delivery. Informational material about cord blood banking is made available through obstetricians and in-hospital obstetrical units. Unfortunately, there are many women who receive little or no prenatal care. Thus, alternatively, the cord blood could be collected and consent obtained from the mother after delivery. We have used a phased consent process

Table 18.6 Issues to be considered in developing cord blood banks.

Informational material provided
Obtaining consent
Determination of the suitability of cord blood for placement in an allogeneic bank
Donor medical history
Donor laboratory testing for infectious diseases
Laboratory testing for genetic diseases
Contamination of CBSCs with maternal blood
Cord blood collection and preservation
Collection
Containers for cord blood
Anticoagulant for short-term storage
Short-term storage conditions
Freezing and long-term storage
Red cell depletion or other processing
Histocompatibility testing
Transplant-related testing specimens
Data, information, and labeling
Transportation conditions
Cord blood testing for suitability for transplantation
Confidentiality

[77]. Consent of the father is not necessary [78]. In addition to obtaining consent for placing the CBSCs in a bank, consent must be obtained for testing the CBSCs and testing the mother for transmissible diseases.

Determination of the suitability of cord blood for placement in an allogeneic bank

A combination of a medical history and laboratory tests [75, 76, 79] should ensure that CBSCs placed in a bank will be as safe as possible and will provide long-term normal hematopoiesis after transplantation. The medical history and laboratory tests used for allogeneic blood donors can be used as a model, since they address the issues of maximum safety and normal blood cell function. A younger maternal age, larger birth weight, shortened gestational age, and shorter time from collection to processing seem to be associated with a higher CD34+ cell concentration [80].

In hematopoietic stem cell transplantation, in addition to minimal transmissible disease risk, the cells must provide a long-term proliferative potential of normal cells. Thus, the medical history and laboratory tests must ensure the absence of hematopoietic stem cell defects. Blood donor requirements include those designed to protect the safety of the recipient and those to protect the safety of the donor. The requirements that protect the safety of the recipient should be used, while those that protect the safety of the donor are modified because of the different donation situation under which cord blood is obtained. In addition, a history of inherited diseases in the mother's and father's families and some unique criteria relating to the pregnancy and delivery are included.

Donor laboratory testing is carried out on blood of the infant and the mother, but the tests are different. In any disease involving viremia in the mother, there is the potential that the virus can cross the placenta and the cord blood may also be viremic. Examples are varicella, cytomegalovirus, hepatitis B, and human immunodeficiency virus. The tests are the same as those currently done for blood donors, since that group of tests is selected for the same reasons as testing of cord blood, namely to minimize the risk of transmitting an infectious disease. If a test is positive, the mother and proper public health authorities must be notified so that early treatment can be initiated; if indicated, steps should be taken to minimize the transmission of disease to contacts of the infant or mother. UCB contains some maternal blood cells, and these could cause GVHD in the transplant recipient. However, in the absence of additional data, routine testing for contaminating maternal blood is not done [45, 81]. Transmission of a genetic disease of hematopoietic stem cells also could occur. Hemoglobin screening of newborns is a routine public health procedure and results are obtained by cord blood banks. If the cord blood is to be used to transplant other inherited disease of hematopoietic stem cells, the blood is tested for that specific disease.

Cord blood collection and preservation

The cord blood can be collected in the delivery room immediately after the cord is clamped and while the placenta remains in the uterus or after delivery when the placenta is taken to a nearby laboratory for collection. In

either case, the umbilical vein is cannulated and the cord blood is drained and collected into a plastic bag system such as those used to collect whole blood [79, 81, 82]. A device involving perfusion of the placenta with solution similar to that used for donor organ perfusion seems to allow collection of adequate doses of CBSCs up to 39 hours after delivery [83]. Both acid-citrate-dextrose (ACD) and citrate-phosphate-dextrose (CPD) have been used successfully as anticoagulants [17, 44, 79, 81]. Cord blood can be collected at remote locations and shipped to a central laboratory for processing without significant loss of colony-forming unit (CFU) and viable CD34+ cells [84].

Red cell depletion or other processing

It is desirable to purify the UCB by removing red cells to avoid hemolysis in cases of donor–recipient ABO incompatibility and to reduce the volume of cord blood that must be stored, although not all banks do this [85]. Most of the stem cells are recovered in the mononuclear cell fraction. Hydroxyethyl starch sedimentation is the most commonly used method to prepare mononuclear cells from UCB [85] but other methods are available [86] including semi-automated devices for the processing and even addition of the cryoprotectant [87]. Reducing the volume of stored material may reduce the costs of storage and the volume of DMSO transfused, and thus should reduce the frequency and severity of reactions [25, 88]. In the future, it may be that a procedure for the positive selection of CD34 cells will also provide the desired RBC depletion.

The cord blood usually will not be frozen immediately after collection, since deliveries occur at all hours and in locations removed from the cell processing laboratory. UCB can be stored for at least 24 hours at either 4°C or room temperature [17, 18, 89, 90]. This is discussed in more detail in the section on preservation of hematopoietic stem cells.

Storage conditions for cord blood

The DMSO and controlled-rate freezing used for bone marrow stem cells can also be used to freeze CBSCs [79, 81, 86]. Although the long-term storage conditions for cord blood are not established, it appears that using controlled-rate freezing and the cryoprotectant 10% DMSO, UCB can be stored in liquid nitrogen (approximately −185°C) for up to 15 years. Thawing and preparing the cord blood for infusion is not standardized. Often the unit is washed [91], although other processes have been proposed [92].

18.6 Adoptive immunotherapy

Adoptive immunotherapy refers to the strategy of altering the patient's immune function using the patient's own or allogeneic mononuclear cells, usually lymphocytes, that are treated in vitro to expand their number or activate the desired function. Adoptive immunotherapy has been used to treat malignancy, viral diseases, diseases with altered immunity, or autoimmune diseases.

Antitumor immunotherapy

The discovery that T-cell growth factor, later renamed interleukin-2 (IL-2), caused the production of cells with antitumor effect led to a substantial amount of work using lymphokine-activated killer (LAK) cells clinically, and this was probably the first attempt at adoptive immunotherapy. When adoptive immunotherapy has been used to treat malignancies, the patient's lymphocytes are collected by apheresis, then the peripheral blood lymphocytes are cultured to increase their number and are activated with cytokines. These lymphokine-activated tumor killer cells are transfused to the original cell donor, possibly with other cytokines. Initial responses rates were encouraging but were associated with serious toxicity [93].

Natural killer and activated natural killer cells

Natural killer (NK) cells are lymphocytes that spontaneously kill tumors without major histocompatibility complex (MHC) restriction. They are important in immune surveillance against malignancy and virus-infected cells [94]. Certain interleukins induce proliferation and activation of NK cells, and this has made it possible to generate large numbers of NK or activated NK cells ex vivo for clinical use. Considerable experience using activated NK cells is promising [94–96].

Peripheral blood mononuclear cells: donor leucocyte infusion

An immunologic graft-versus-leukemia effect is provided by peripheral blood mononuclear cells [97–104]. Transfusion of allogeneic mononuclear cells from the original bone marrow donor or donor leukocyte infusion (DLI) appears to provide a graft-versus-leukemia effect in acute myelogenous leukemia (AML), chronic myelogenous leukemia, acute lymphocytic leukemia (ALL), and other hematologic malignancies [102]. Despite the rather large number of T cells transfused (approximately 11×10^8/kg), GVHD is somewhat more mild than expected. If mixed chimerism is present for instance from using a nonmyeloablative preparative regimen, the antitumor effect of DLI is maintained with mild to modest GVHD [103]. It appears that DLI may aid in hematopoietic cell graft survival by inducing donor-specific tolerance through the recipient's regulatory T cells [104]. Thus, leukocytes collected by apheresis from the marrow donor are transfused as a mononuclear cell concentrate—a new blood component for DLI.

Epstein–Barr virus lymphoproliferative disorders

Epstein–Barr virus (EBV) specific T-lymphocytes can be generated ex vivo and large numbers produced for immunotherapy [105]. EBV is associated with Hodgkin's disease and can cause a lymphoproliferative disease in immunocompromised patients. Lymphocytes from the donor or patient can be primed against viral (EBV) antigens, cultured to increase their number, and transfused to provide anti-EBV therapy [106–108]. Posttransplant EBV-induced lymphoproliferative disorder can be successfully treated by transfusions of lymphocytes obtained from the

original bone marrow donor [109, 110]. Approximately 1×10^6 T cells per kilogram are transfused, thus constituting another new blood component.

Dendritic cells

Dendritic cells are the most potent antigen-presenting cells with the unique ability to initiate and maintain primary immune responses when the dendritic cells have been stimulated with antigen. Dendritic cells originate in the bone marrow and migrate via blood to most organs where they are present in an immature state. Dendritic cells have a high rate of antigen uptake. When dendritic cells are exposed to a tumor- or viral-associated antigen, a specific set of cytotoxic T cells are stimulated, these cytotoxic T-cells can then be isolated and cultured to increase their number and used as an antitumor therapy. Thus, dendritic cell "vaccination" is now receiving much attention as an exciting new therapy [111–115]. Immunotherapy with dendritic cells usually is an autologous process using tumor antigen material obtained from the patient along with that patient's dendritic cells to generate a large number of cytotoxic T lymphocytes specifically directed against that patient's own tumor antigen. The dendritic cells can be obtained from mononuclear cell concentrates obtained by leukopheresis [116–118]. A number of studies of dendritic cell immunotherapy are beginning to be reported but because they use different tumor antigens, different diseases, and different methods of stimulating the dendritic cells with antigen and most results are very preliminary. Dendritic cell immunotherapy will be a major area of investigation in cancer therapy during the next several years, and the methods of stimulating the dendritic cells and generating the cytotoxic T-cell clones will be important activities in cellular engineering. One dendritic cell-related therapy for immune therapy of metastic prostate cancer is now licensed by the FDA. PBSCs are collected by apheresis and dendritic cells are isolated. Autologous antigen presenting dendritic cells are cultured with a fusion protein of prostatic acid phosphatase linked to G-CSF which results in inactivation and up regulation of co-stimulatory molecules [119, 120]. Large-scale clinical trials have demonstrated a modest, prolonged time to cancer recurrence [119, 120].

T-regulatory cells

An important part of maintaining immune tolerance is T-cell-mediated immune suppression toward self and nonself antigens. T-regulatory cells, previously known as T-suppressor cells, are fundamental to the control of the immune response. Absence or defective T-regulatory function is associated with autoimmune disease and restoration of T-regulatory function restores tolerance. Adoptive immune therapy with T-regulatory cells is just beginning [121–123]. T-regulatory cells can be obtained from mononuclear cell products collected by apheresis. The autologous T-regulatory cells are isolated, then cultured to increase the number and transfused to the patient. Initial use is for treatment or prevention of GVHD in hematopoietic stem cell transplant patients. If successful,

T-regulatory cell therapy could be attempted in solid organ or autoimmune diseases [121–123].

Antiviral immunotherapy
Cytomegalovirus
Cytomegalovirus (CMV) infection and disease is a major cause of morbidity and mortality in patients undergoing hematopoietic cell transplant (HCT). Clones of lymphocytes with specific anti-CMV cytotoxicity can be generated ex vivo [124–126] and used to restore CMV immunity in HCT patients [125, 127, 128].

Epstein–Barr virus
Use of T-cell clones to treat lymphoproliferative disease due to EBV is described in the section on malignant disease.

Immunotherapy for immune diseases
Autoimmune diseases
Type I diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, and multiple sclerosis, all result in decreased suppressor T-cell activity. In type I diabetes, the anti-beta cytotoxicity appears to be due to lymphocyte-mediated cellular cytotoxicity. A few type I diabetes mellitus patients have been treated with lymphocyte transfusions, but experience is very limited and a clinical benefit of this approach has not been established [93]. It is exciting to speculate that type I diabetes mellitus and other autoimmune disorders might be amenable to transfusion therapy using suppressor T cells.

Recurrent spontaneous abortions
Some patients may experience recurrent spontaneous abortion because of immunologic factors. It has been postulated that a successful pregnancy depends on maternal recognition of paternal alloantigens followed by suppression of reactivity against crossreactive antigens in the trophoblast [129]. This may be due to high NK cell activity because reduction of NK activity improves the likelihood of maintaining a successful pregnancy. Immunization of women with paternal mononuclear cells reduces the rate of spontaneous abortion in these patients [130], although this has not become a generally used therapy.

18.7 Mesenchymal stem cells

These cells were originally found in the bone marrow and were thought to support hematopoiesis. Subsequently, it was shown that they have the capacity to differentiate into a wide variety of mesenchymal tissues [131] and thus have the potential to be a cell source for regenerative medicine. Currently mesenchymal stem cells (MSCs) are a very active area of research involving their use to facilitate hematopoietic stem cell engraftment [132],

treat GVHD [133], abdominal fistula [134], osteogenesis imperfecta [135], amyotrophic lateral sclerosis [136], and myocardial repair.

18.8 Myocardial repair

In addition to MSCs, cardiac progenitor cells can be obtained from the heart and marrow or blood CD34+ cells can be differentiated into myocardial cells [137–140]. This is a major area of clinical and laboratory investigation and opens the possibility of restoration of cardiac function by cellular therapy.

18.9 Regulation of cellular engineering

The Code of Federal Regulations (CFR) defines a biological product as one containing some organic constituent derived from whole blood, plasma, or serum [141]. These biological materials are regulated under US Food and Drug Administration (FDA) law. The FDA has also stated its intention to regulate somatic cell therapy [142]. The FDA considers that somatic cell therapy products are autologous or allogeneic cells that "have been propagated, expanded, selected, pharmacologically-treated, or otherwise altered in biological characteristics ex vivo to be administered to humans and applicable to the prevention, treatment, cure, diagnosis, or mitigation of disease" [142]. Such somatic cell biological products are "subject to establishment and product licensure to ensure product safety, purity, and potency" [142]. Clinical trials involving FDA-regulated products must be conducted under an IND application.

The FDA has clearly stated [142] that the following kinds of cells will be subject to FDA licensure: (a) "autologous or allogeneic lymphocytes activated and expanded ex vivo . . ."; (b) "encapsulated autologous allogeneic or xenogeneic cells intended to secrete a bioactive factor . . ."; (c) "autologous or allogeneic somatic cells . . ."; (d) "cultured cell lines . . ."; and (e) "autologous or allogeneic bone marrow transplants using expanded or activated bone marrow cells." Cells for which FDA approval is not currently required include "minimally manipulated or purged bone marrow transplants . . . including . . . allogeneic BMT employing ex vivo T-cell purging with a monoclonal antibody approved for such purging, autologous BMT employing ex vivo tumor cell purging by an approved agent, and autologous BMT employing bone marrow enriched for stem cells by immuno adherence." More recently, the FDA definition of minimal manipulation as applied to hematopoietic cell processing includes cell separation, cryopreservation, and freezing [143]. The extent of regulation of cell processing will be based on whether the cells are (a) more than minimally manipulated, (b) used for their normal homologous function, (c) combined with noncell or nontissue products, or (d) used to provide a metabolic function [143].

Thus, it is clear that the collection and processing of marrow or blood cells for the development of novel cellular therapies is an FDA-regulated function. The FDA expects this activity to be carried out in conformance with their regulations regarding the manufacture of biological or pharmaceutical products. Most cell-processing procedures are used in the equivalent of phase I or II clinical trials. Many cell-processing procedures will never become routinely used on a scale that would warrant licensure. However, if the procedures involve more than minimal manipulation, they must be done under an IND application. Many of these procedures also involve devices or reagents that will be under IND applications or investigational device exemptions (IDEs). Thus, there are extensive regulatory requirements and involvement in cell-processing programs. The reagents will have to be produced in appropriate facilities in a manner suitable for human (not laboratory) use; the starting material (marrow, blood cells) will have to be obtained in accordance with proper medical and laboratory screening procedures and processed in a properly controlled facility using containers and reagents intended for human use.

CBSCs are also regulated by the FDA. These cells are mentioned along with marrow and peripheral blood as sources of stem cells in the FDA description of the regulatory approach for cells undergoing more than minimal processing or being used for unrelated allogeneic transplants [143]. Thus, an IND application is required for cord blood even for related-donor transplants including all of the appropriate consents, record keeping, and approval mechanisms. The FDA has indicated the intention to require licensure for cord blood effective October 2011.

18.10 Quality assurance and good manufacturing practices for cellular engineering

Because of the dramatic increase in the complexity and diversity of the new cellular products, the FDA regulation of the components, and the production and use of the components under IND applications, it has become essential to develop effective quality assurance programs for cell processing [144, 145].

Despite the developmental and evolving nature of cellular engineering, it is possible to develop quality assurance systems that are supportive of and consistent with these laboratory activities. The general issues and approaches to quality assurance programs in transfusion medicine are described in Chapter 20. The major components of a quality assurance program are the adherence to good manufacturing practices (GMPs) and the development and use of critical control points. When a pharmaceutical or biological agent has been licensed and is being produced commercially on a large scale, the GMPs are extensive, the facilities are complex, and quality assurance systems are comprehensive. At the present relatively immature stage of somatic cell processing, products are used in small numbers as part of experimental therapeutic programs and early stage clinical trials.

The quality assurance and GMP process is intended to provide for the safety, purity, and potency of the product [141, 144–146]. These are essential qualities of somatic cell products even during phase I or II trials. It is important to ensure that the product is as safe as possible, does not contain any unintended materials (chemicals, infectious agents, cells), and does contain the desired amount of the therapeutic agent (cells). GMPs that are appropriate to somatic cell processing must be used so that the products will be as standard as possible to maximize the likelihood that the therapeutic outcome—including any adverse effects—can be clearly related to the product. In this way, investigators can more easily determine the cause of any unexpected adverse effects, and the therapeutic benefit (or lack thereof) of the product can be attributed to the therapeutic strategy, not a failure in the product potency.

The eight parts or components of GMPs as defined in the CFR include the: (a) organization and requirements for personnel responsible for different aspects of the operation; (b) the buildings and facilities necessary for different aspects of the operation; (c) equipment; (d) the production and process control requirements; (e) requirements for controlling and releasing the finished product; (f) laboratory controls; (g) the records and reports that should be maintained; and (h) general stipulations. A quality control program for any biological agent should include control of the biological source, the production process, and the final product. The laboratory must have a complete, up-to-date, written procedure manual readily available and understood by the staff. The staff must have the skills necessary to carry out their tasks, training programs must be in place, and there must be evidence that personnel are proficient at these tasks. Ideally, standardized work sheets should be completed as the cord blood is processed, and they should be designed in a step-by-step style or format. Electronic records are preferable but not yet well developed. The laboratory should also establish a mechanism to ensure that all necessary data are recorded and that the data meet preset criteria. There should be a mechanism to carry out structured calculation or formulas in a worksheet format, including a system to double-check all calculations. The data describing the final product and tests at intermediate steps should be recorded. The identity of all personnel who are involved in the procedures must be recorded. The conditions of storage must be recorded, and any deviations should be documented and explained.

After frozen storage, the hematopoietic stem cell component must be prepared for transfusion. Important considerations at this stage include the rate of thawing, postthaw storage temperature and duration, and the method of transfusion. Procedures must be in place for all of these steps. A report containing pertinent information about the cells administered should be included in the patient's chart. A system of monitoring deviations from operating procedures has been proposed [145].

The equipment that would be part of a stem cell processing facility includes laminar air flow hoods, centrifuges, programmable freezers, storage containers for frozen stem cells, alarms, liquid nitrogen supply, refrigerators/freezers for storage of reagents and media, water baths, and

CO_2 incubators. Procedures for testing each piece of equipment must be established, the frequency of such tests described, and acceptable limits set.

The development and application of GMPs to somatic cell processing must take into account the variety and continually evolving nature of the procedures. Progenitor-cell-processing procedures that are currently in use may involve one or more variations of methods for volume reduction, red cell depletion, T-cell depletion of bone marrow, positive selection of stem cells (CD34+), purging marrow of malignant or other cells, preparing buffy coats, freezing and storing cells, clustering or activating cells, or other complex steps. There may be several variations of these methods, and each may be carried out on marrow, peripheral blood, or cord blood as the starting material. Thus, the GMPs must take this variety into account.

These somatic cell, hematopoietic progenitor, and regenerative medicine products are still quite developmental and undergo change based on research developments. However, GMPs can provide a structure that provides quality but allows the developing, evolving nature of somatic cell processing.

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19 Therapeutic Apheresis

The pathophysiology or symptoms of some diseases are due to the excessive accumulation of blood cells or plasma constituents. In these situations, blood cell separators ordinarily used to collect blood components by apheresis from normal donors can also be used therapeutically [1]. In the United States, in 2006, approximately 112,109 therapeutic apheresis procedures were performed, about 20% by blood centers and 80% by hospitals [2]. The specific indications reported most commonly by facilities were thrombotic thrombocytopenic purpura (TTP) (23% of all procedures) followed by hemochromatosis(15%), myasthenia graves (10%), Gillian-Barre syndrome, multiple sclerosis, sickle cell disease, chronic inflammatory demyelinating polyradiculoneuropathy, and Good Pasture's syndrome [2]. About 70% of therapeutic apheresis procedures are plasma exchange [3]. The Canadian Apheresis Study Group reports that therapeutic apheresis procedures are performed at a rate of about 6000 per year, or 23 procedures for each 100,000 of the population [4]. While plasma exchange for TTP, a hematologic disease, is the most common therapeutic apheresis, overall the procedure is probably done predominantly for neurologic diseases, primarily because of the use of therapeutic plasma exchange (TPE) for the treatment of Guillain–Barré disease [4, 5].

19.1 Clinical uses of plasma exchange

Plasma exchange is the most common form of therapeutic apheresis. The diseases in which plasma exchange is used can be categorized by the type of disease or the type of material being removed [3, 6–8] (Tables 19.1, 19.2).

Some of the diseases in which plasma exchange is used are quite well understood, the constituent being removed is known, and good clinical studies have established the value of plasma exchange therapy. In many other situations the pathophysiology of the disease is poorly understood, there may not be specific laboratory measurements of a known pathologic agent that can be correlated with improvement in the patient's clinical condition following plasma exchange, or well-designed clinical trials have not been done. Clinical fluctuations or spontaneous improvements occur in many of these diseases, making the benefits of plasma exchange difficult to establish. The American Medical Association (AMA) developed indication categories for therapeutic apheresis [6]

Table 19.1 Type of disease in which therapeutic plasma exchange may be used.

Hematologic
Neurologic
Renal
Transplant
Collagen vascular
Dermatologic
Metabolic

(Table 19.3). Periodically, the American Society for Apheresis (ASFA) and the American Association of Blood Banks update the situations in which therapeutic apheresis is being used and categorize them according to the AMA categories (Table 19.4) [9, 10].

Neurologic diseases

It is estimated that half of the 20,000–30,000 plasma exchange procedures performed in the United States are done to treat neurologic diseases [6], which probably accounts for the largest group of plasma exchange procedures. Usually six procedures are done in about 2 weeks. For some situations, such as Guillain–Barré syndrome and Goodpasture's syndrome, more intensive therapy is helpful.

Guillain–Barré syndrome

For Guillain-Barré syndrome, plasma exchange shortens the duration of motor weakness, reduces the hospital stay, and reduces the period of ventilation dependence in patients who can breathe on their own at the time treatment is initiated but subsequently require ventilatory assistance [5, 11, 12]. Plasma exchange therapy is more effective if (a) it is performed early in the disease, (b) it is applied to rapidly progressive disease, and (c) there is an absence of other general contraindications such as infection, cardiac arrhythmia, myocardial insufficiency, or coagulation disorders [13]. Personal experience suggests that plasma exchange is more effective if used very early; therefore, therapy should be initiated within 12 hours of recognizing the problem and be continued daily for several days. Low-amplitude muscle action potential, advanced age, longer time from the onset of disease, and increased need for ventilatory support are correlated with poorer outcome of plasma exchange [14]. Intravenous

Table 19.2 Type of material being removed by plasma exchange.

IgG
IgM
Abnormal proteins
Immune complexes
Excess normal substance
Replace deficient normal substance

Table 19.3 Indication categories for therapeutic apheresis.

Standard therapy, acceptable but not mandatory
Available evidence tends to favor efficacy; conventional therapy usually tried first
Inadequately tested at this time
No demonstrated value in controlled trials

Source: Council on Scientific Affairs. Current status of therapeutic plasmapheresis and related techniques—report of the AMA panel on therapeutic plasmapheresis. JAMA 1985; 253:819–825.

Table 19.4 Indication categories for therapeutic apheresis.

Disease	Category
Renal diseases	
Acute hepatic failure	III[a]
ANCA-associated rapidly progressive glomerulonephritis	II[a], II[a], III[a]
Anti-glomerular basement membrane disease	I[a], I[a], IV[a]
Hemolytic uremic syndrome	II[a], I[a], IV[a]
Focal segmental glomerulosclerosis	I[a]
Wilson's disease, fulminant	I[a]
Immune complex rapidly progressive glomerulonephritis	III[a]
Renal transplantation	
Antibody-mediated rejection	I[a]
HLA desensitization	II[a]
High PRA, cadaveric donor	III[a]
Metabolic diseases	
Familial hypocholesterolemia	I[b], II[b], II[a]
Hereditary hematochomatosis	III[c]
Hypertriglyceridemic pancreatitis	III[a]
Nephrogenic systemic fibrosis	III[d], III[a]
Overdose, venoms, and poisoning	II[a], III[a], III[a], III[a]
Phytanic acid storage disease	II[a]
Sepsis with multiorgan failure	III[a]
Thyroid storm	III[a]
Autoimmune and rheumatic diseases	
Catastrophic antiphospholipid syndrome	II[a]
Cryoglobulinemia	I[a], II[e]
Inflammatory bowel disease	II[f]
Pemphigus vulgaris	IV[a], III[d]
Rheumatoid arthritis	II[e], IV[a]
Scleroderma	III[a], IV[d]
Systemic lupus erythematosus	III[a], IV[a]
Hematologic Diseases	
ABO-mismatched hematopoietic progenitor cell transplant	II[a]
Aplastic anemia; pure RBC aplasia	III[a], II[a]
Autoimmune hemolytic anemia; cold agglutinin disease	III[a], II[a]
Babesiosis	I[c], II[c]
Coagulation factor inhibitors	III[e], IV[a]
Cutaneous T-cell lymphoma	I[b], III[b]
Erythrocytosis and polycythemia vera	III[c], III[c]

(Continued)

Table 19.4 (*Continued.*)

Disease	Category
Graft-versus-host disease (skin; nonskin)	II[d], II[d], III[d]
Hyperleukocytosis	I[g], III[g]
Hyperviscosity in monoclonal gammopathies	I[a], I[a]
Malaria	II[a]
Myeloma cast nephropathy	II[a]
Posttransfusion purpura	III[a]
Red cell alloimmunization in pregnancy	II[a]
Sickle cell disease	I[c], II[c], II[c], III[c]
Thrombocytosis	II[h], III[h]
Thrombotic microangiopathy	
Drug-related	I[a], III[a], IV[a], IV[a]
HSC transplant-related	III[a]
Thrombotic thrombocytopenic purpura	I[a]
Transplantation-related	
ABO-mismatched solid organ transplant (kidney, liver)	II[a], III[a]
Dilated cardiomyopathy	III[e], III[a]
Heart transplant rejection	I[d], II[d], III[a]
Lung allograft rejection	II[d]
Neurologic disorders	
Acute disseminated encephalomyelitis	II[a]
Acute inflammatory demyelinating polyneuropathy	I[a]
Chronic inflammatory demyelinating polyradiculoneuropathy	I[a]
Lambert–Eaton myasthenic syndrome	II[a]
Multiple sclerosis	II[a], III[a]
Myasthenia gravis	I[a], I[a]
Neuromyelitis optica	II[a]
Paraneoplastic neurologic syndromes	III[a], III[e]
Sydenham's chorea	I[a]
Paraproteinemic polyneuropathies	
IgG/IgA	I[a]
IgM	I[a]
Multiple myeloma	III[a]
IgG/IgA or IgM	III[e]
PANDAS+	I[a]
Rasmussen's encephalitis	II[a], II[e]
Stiff-person syndrome	IV[a]

Source: Szczepiorkowski ZM, Winters JL, Bandarenko N, et al. Guidelines on the use of therapeutic apheresis in clinical practice—evidence-based approach from the Aphersis Applications Committee of the American Society for Aphersis. J Clin Apher 2010; 25:83–117.

I, therapeutic apheresis (TA) standard as primary therapy or first-line adjunct to primary therapy; II, TA accepted as adjunct to primary therapy; III, suggestion of benefit of TA with insufficient evidence to determine efficacy or cost/benefit; IV, controlled trials have not shown benefit of TA; PANDAS, pediatric autoimmune neuropsychiatric disorders.

[a]Plasma exchange.
[b]Selective removal.
[c]Erythrocytapheresis.
[d]Photopheresis.
[e]Immunoadsorption.
[f]Adsorptive cytapheresis.
[g]Leukocytapheresis.
[h]Thrombocytapheresis.

immune globulin is as effective as plasma exchange and this is now commonly used along with plasma exchange [15–17], or alone if plasma exchange is not available or a practical option.

Myasthenia gravis

Plasma exchange has been used to lower the level of IgG-type antibodies to the acetylcholine receptor that are an important part of the pathogenesis of myasthenia gravis [18, 19]. There are many anecdotal reports of clinical improvement following plasma exchange in patients with myasthenia gravis including use of the staphylococcal protein A (SPA) column [5, 20]. However, no controlled trial of plasma exchange has ever been reported. It appears that plasma exchange results in improvement in about two-thirds of patients, usually within 1–3 days of the start of the therapy [19]. Therefore, plasma exchange is recommended but only in the early stage before other treatments have become effective, in a crisis, or in chronic cases as an adjunct to other forms of immunosuppressive therapy [5, 20].

Multiple sclerosis

Because immunologic processes play an important role in multiple sclerosis, there has been interest in the use of plasma exchange to alter the humoral immunity in these patients. One clinical trial comparing plasma exchange with sham exchange found improvement in 28 of 45 patients in the plasma exchange arm [21]. There are few results from other structured trials. Because most patients with multiple sclerosis have a gradual downhill course but survive for 25 years, the Consensus Conference [5] concluded that "a treatment as uncomfortable, time-consuming, and expensive as plasma exchange is unlikely to be appropriate." It was also believed that plasma exchange would not be effective for patients who have already developed substantial neurologic damage. Thus, the use of plasma exchange might be beneficial in the approximately 5% of patients who develop a malignant course with severe disability in about 5 years [5]. However, plasma exchange is not recommended as a part of the therapy for multiple sclerosis [5, 22–25].

Lambert–Eaton syndrome

Lambert–Eaton syndrome is a rare disorder that resembles myasthenia gravis. It is probably caused by an antibody to calcium channels in the myoneural junction. Because of the immunologic pathogenesis of the disease and a few individual reports suggesting that patients are improved by plasma exchange, plasma exchange is recommended as a part of the treatment of Lambert–Eaton syndrome [5, 26].

Chronic inflammatory demyelinating polyradiculoneuropathy

One controlled study comparing plasma exchange with sham apheresis demonstrated a benefit for plasma exchange in this disease. However, the

Consensus Conference considered that the overall benefit was probable rather than proven [5]. About one-third of the patients in the trial showed an improvement. Plasma exchange is used as initial therapy for those who cannot walk and as alternative therapy for those who cannot be tapered from steroids.

Monoclonal gamopathies

Neurologic impairment can occur in patients with paraproteinemias. Reduction of the level of the paraproteins by chemotherapy or plasma exchange may improve the neurologic symptoms [27], but large-scale studies and extensive data to support this are not available. Thus, plasma exchange can be used as a part of the treatment strategy in these diseases, but data are not available to establish its value.

Amyotrophic lateral sclerosis

The cause of amyotrophic lateral sclerosis (ALS) is not known, and plasma exchange has not been shown to be of benefit in treating this disease.

Renal diseases
Goodpasture's syndrome

Goodpasture's syndrome is a rapidly progressive syndrome in which renal damage is caused by antiglomerular basement membrane antibodies. Plasma exchange has been effective in slowing or reversing the renal and pulmonary damage [28–30]. Plasma exchange seems to be more helpful if it is instituted early in the course of disease [31]. If pulmonary hemorrhage is present, we consider this to be a situation requiring urgent therapy and would institute plasma exchange within about 12 hours. If renal function is deteriorating, plasma exchange can be done in conjunction with hemodialysis. We would carry out plasma exchange frequently, almost daily for the first few days, on the rationale that early aggressive therapy is the most likely to be beneficial.

Rapidly progressive glomerulonephritis

This is a condition sometimes associated with antinuclear cytoplasmic antibodies or immune complexes in which the causative antigen is not known. The role of plasma exchange in this situation is not clear. Plasma exchange may be more beneficial in patients with antinuclear cytoplasmic antibodies and is sometimes used as initial therapy in combination with immunosuppressive drugs [31, 32].

Multiple myeloma

Although multiple myeloma is a hematologic disease, renal damage occurs in a high proportion of patients, and survival is short once renal failure occurs. Light chains are thought to play a major role in the renal damage. Several studies have reported that plasma exchange is effective in removing light chains and improving renal function [33–36]. A controlled

trial of plasma exchange in patients with renal failure due to myeloma was rather dramatic, with 13 of 15 plasma exchange patients recovering renal function compared with only 2 of 11 in the control group [37]. The 1-year survival was 66% in the plasma exchange group and 28% in the control group. Thus, plasma exchange, along with diuresis and chemotherapy, is probably helpful in the management of renal damage in multiple myeloma.

Hematologic diseases

Thrombotic thrombocytopenic purpura

TTP is associated with a severe deficiency of von Willebrand factor cleaving protease (ADAMTS 13) resulting in high levels of large vWF multimers and microvascular platelet thrombi [38]. The application of plasma exchange and probably better overall care has resulted in survival rates of 70–80% [39–43]. Plasma exchange may act by depleting high-molecular-weight vWF multimers, by replacing ADAMTS 13, or both. The level of ADAMTS 13 may predict the result of plasma exchange [44] but this assay is only available in a research setting and is not specific for TTP [45, 46].

Plasma exchange is a major part of the therapy of TTP [47]. It should be initiated early in the disease and continued daily, if necessary, to increase the platelet count. Because of the need to readjust the high- and low-molecular-weight vWF multimers and/or replace ADAMTS 13, plasma must be used as the replacement solution. Solvent–detergent plasma that is deficient in protein S should not be used as a replacement fluid as this may lead to deep vein thrombosis [48]. Either fresh frozen or cryoprecipitate-poor plasma (lacking high-molecular-weight multimers) can be used initially [49, 50]. If the patient fails to respond, the other form of plasma should be used. When the disease appears to have stabilized with increasing platelet counts and decreasing levels of lactate dehydrogenase (LDH), the frequency of plasma exchange can be reduced gradually—for instance, to every other day. Some patients may require a large number of plasma exchanges to maintain their platelet count. One man we have treated required more than 150 plasma exchanges over 2 years before he ultimately resolved his disease. These patients are often quite ill, and 30% [51] or more have major complications or death associated with plasma exchange. Serious complications include hemorrhage or pneumothorax due to catheter placement, systemic infection, thrombosis, or hypoxemia and hypotension [51].

Cryoglobulinemia

Cryoglobulinemia with symptoms due to abnormal IgM can be treated with plasma exchange. Neurologic or renal complications or Raynaud's phenomenon have been improved by plasma exchange [52, 53]. Because IgM is primarily intravascular, plasma exchange is very effective in lowering the levels. The underlying disease must be treated, but plasma exchange can be very helpful in dealing with acute problems caused by the cryoglobulins.

Cold agglutinin disease

The symptoms and hemolysis associated with high levels of cold agglutinins can be improved acutely with plasma exchange [54]. As with cryoglobulinemia, the underlying disease must be treated, but plasma exchange is an effective strategy to reduce the level of IgM antibody and reduce the hemolysis [54].

Hyperviscosity syndrome

Hyperviscosity syndrome is usually caused by IgM-type immunoglobulins, and plasma exchange is very effective in lowering IgM, reducing (improving) viscosity, and relieving symptoms. Plasma exchange can be a valuable part of the acute treatment of hyperviscosity while measures such as chemotherapy take effect.

Coagulation factor inhibitors

Plasma exchange has been used to lower the level of inhibitor to deal with a crisis or urgent surgery [55], but more contemporary approaches to the management of inhibitors have made plasma exchange less valuable. A column for selective IgG removal has been used [56], but this device is not licensed for use in the United States.

Catastrophic antiphospholipid syndrome

Because this syndrome is associated with autoantibodies to phospholipids, plasma exchange has been considered. Usually CAPS responds to anticoagulation but when complications such as pulmonary hemorrhage preclude anticoagulation, plasma exchange may be helpful [57], although this is not one of the ASFA recommended indications.

Autoimmune thrombocytopenia

Although idiopathic thrombocytopenic purpura (ITP) is due to platelet autoantibodies, strategies other than plasma exchange are used.

Alloimmune platelet refractoriness

Plasma exchange including use of the SPA column has been used but has not been shown to be helpful (see Chapter 11).

Posttransfusion purpura

Because posttransfusion purpura (PTP) is an antibody (and/or immune complex) disease, plasma exchange can be effective [58, 59]; however, use of antigen-negative platelets or intravenous immunoglobulin (IVIG) is preferred (see Chapter 12).

Solid organ transplantation
Rejection of transplanted organs

Plasma exchange has been attempted in the belief that antibody is involved in rejection, but it is not possible to remove enough antibodies quickly to be effective [60–62] and ultimate graft survival is not improved [60].

Pretransplant

Recently, interest has developed in using plasma exchange to lower HLA antibody titers in immunized patients in hopes of avoiding early antibody-mediated rejection [60, 63, 64]. Usually treatment involving daily or even twice daily exchanges for several days is necessary, but uncontrolled studies seem to indicate that this has facilitated successful transplantation. Plasma exchange has also been used to reduce A or B antibodies to enable ABO incompatible kidney, liver, or heart transplants [60]. Because of the difficulty in lowering anti-A or B, patients should have starting antibody titer less than 1 : 128. If the titer can be lowered to 1 : 4, it appears that transplants can be successful [60, 65–67]. Plasma exchange for both HLA immunized and ABO incompatible patients is very labor intensive, complex, and requires close collaboration between transfusion medicine and transplant physicians.

Preparation for liver transplantation

Patients awaiting liver transplantation may have coagulopathy or other metabolic complications due to the liver disease. Plasma exchange can be used to stabilize and maintain these patients while they await a donor organ.

Collagen vascular diseases
Rheumatoid arthritis

Rheumatoid arthritis is a chronic autoimmune inflammatory disease of unknown etiology. Because the immunologic alterations are thought to be important in the pathogenesis of the disease, some treatment has involved removing lymphocytes, plasma, or both. Initial studies suggested that a course of lymphoplasmapheresis over about 3 months caused clinical improvement that lasted 3–4 months after therapy was stopped [68]. A later small controlled trial involving sham apheresis also demonstrated reductions in circulating immune complexes, rheumatoid factor, and joint swelling and improvement in clinical function [69]. An accompanying editorial [70] advised caution in interpreting the results and especially in applying this to the general treatment of rheumatoid arthritis. A subsequent controlled trial involving sham apheresis or plasma exchange but no lymphocyte removal did not show a clinical benefit [71]. Patients in the groups receiving plasma exchange and sham apheresis improved equally, suggesting a placebo effect. Several laboratory measures improved, but these changes were not associated with improvements in clinical measures. In the absence of very substantial data defining a clear benefit for plasma exchange in some phase of rheumatoid arthritis, this has not become a part of therapy and is not recommended at present [72].

Systemic lupus erythematosis (SLE)

The presence of autoantibodies and circulating immune complexes suggests that plasma exchange might be helpful in SLE. A controlled trial did not show benefit in lupus nephritis [73] and studies in SLE have mixed results [74, 75]. Thus, plasma exchange is not recommended.

Scleroderma

This is a disease due to overproduction of extracellular matrix proteins. Although these patients may have a variety of autoantibodies, they are not key to the pathogenesis of scleroderma and plasma exchange is not used for therapy.

Other miscellaneous diseases

Pemphigus

IgG autoantibodies directed against intracellular bridges and skin basement membrane may be involved in the pathophysiology of pemphigus. High-dose corticosteroids are effective treatment in most patients, but this therapy requires several weeks to become effective. Plasma exchange has been used in the early acute stage of the disease or for maintenance in patients who respond poorly to corticosteroids. Individual case reports and series have shown a reduction in the IgG autoantibodies and a clinical benefit from plasma exchange [76]. A controlled trial did not show a benefit, and there was no reduction in autoantibody level. Thus, although the data are not conclusive, plasma exchange may be used in the early stages of pemphigus or for patients who fail to respond to steroids.

AIDs-related idiopathic thrombocytopenic purpura

Although plasma exchange has not been used successfully in ITP, it has occasionally been helpful in AIDS-related ITP when combined with IVIG [77]. This was attempted because of the desire to avoid immunosuppressive therapies such as corticosteroids or splenectomy in these patients. However, plasma exchange has not been generally used in this situation. Several reports of the use of the SPA column also appeared promising (see below), but this also has not gained much use and the columns are not available in the United States.

19.2 Plasma exchange

Vascular access for plasma exchange

Vascular access for plasma exchange may be via peripheral veins or by venous catheters. Peripheral venous access is preferable because there are additional complications associated with the use of central venous catheters. Plasma exchange can usually be initiated using antecubital veins, as illustrated by the observation that only 4% of patients were ineligible for one randomized trial because of inadequate peripheral venous access [78]. Unfortunately, venous catheters are often necessary because peripheral veins are too small to accommodate 16–18 gauge needles or are inadequate for the multiple venipunctures necessary for several plasma exchanges. Central venous catheters have been necessary in 50–70% in several studies of plasma exchange in different patient groups [79, 80]. If a catheter is necessary, the apheresis personnel should discuss the venous access needs with the patient's primary physician so that the optimum catheter can be selected. Some considerations are (a) the number of therapeutic apheresis

procedures expected, (b) other treatments that might require venous access, (c) the expected duration of treatment, (d) whether or not the patient will remain in the hospital, and (e) the availability of family members to assist with catheter care, if the patient will be out of the hospital [81]. The types of catheters that have been used most commonly for plasma exchange include Hickman, Quinton–Mahurkar, and triple lumen. The Quinton–Mahurkar catheters may be placed in the jugular, subclavian, or femoral veins. In one study [80], the complication rate was lower with Quinton–Mahurkar catheters than with the other two. The Quinton–Mahurkar catheter has a double lumen, and the outflow and inflow holes at the tip are separated to minimize recirculation [81]. If catheters are to be used, they must have sufficient rigidity so they do not collapse when negative pressure for blood removal is applied. The Quinton–Mahurkar catheter is made of temperature-sensitive material that is rigid during insertion but softens as the temperature increases inside the vein after insertion. It is not advisable to position the return flow from a catheter near the right atrium endocardium because of the possibility that the replacement solution might create an irritable focus and result in cardiac arrhythmia.

Techniques of plasma exchange

In plasma exchange using blood cell separators, the whole blood enters the instrument, where most of the red cells, leukocytes, and platelets are separated from the cell-poor plasma. This plasma is diverted into a waste bag and is replaced with one or more of several available solutions [82]. These include fresh frozen plasma, albumin, and saline.

Several instruments can be used for plasma exchange including: Caridian Spectra, Fenwal CS-3000, Fresenius AS104, and Haemonetics MCS. The Caridian, Fenwal, and Fresenius instruments are continuous flow systems that make it easier to control the patient's fluid volume, while the Haemonetics instrument uses repeated cycles of filling and emptying the blood cell separator. In the United States, the Caridian Spectra is probably the most commonly used instrument. Details of operating these instruments are summarized in McLeod [1], but the manufacturer's operating manuals must be used because of the complexity of these procedures. Thorough quality control programs are essential to be sure that staff are properly trained, fluids monitored, alarms tested, lines and fluid attachments secured, and medications and replacement solutions used correctly. The plasma removed must be discarded as biohazardous waste.

The volume of plasma to be exchanged is usually based on the estimated plasma volume of the patient. Because there is continuous mixing, in the patient, of replacement solution and patient's plasma, the relationship between the fraction of the unwanted compound remaining and the proportion of the patient's plasma volume exchanged is exponential (Figure 19.1). After exchange equal to the patient's plasma volume, the unwanted component will be reduced to approximately 35% of the initial value. Exchanging two times the patient's plasma volume further reduces the unwanted component only to approximately 15% of the initial value.
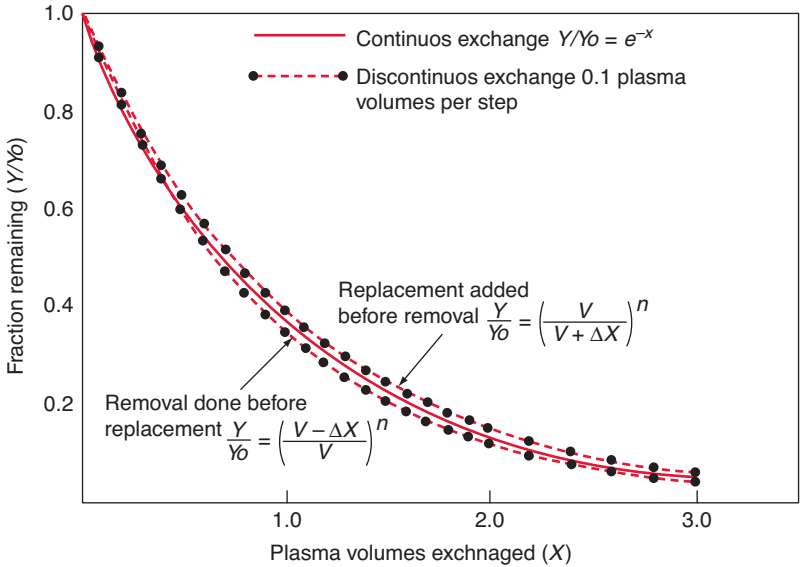
Figure 19.1 Relationship between volume of plasma exchanged and proportion of constituent remaining. (Reproduced with permission from Chopek M, McCullough J. Therapeutic plasma exchange. Lab Med 1981; 12:745–753. © 1981 American Society for Clinical Pathology and © Laboratory Medicine.)

Because of this diminishing effectiveness, usually one or at most 1.5 times the patient's plasma volume is exchanged. Depending on the size of the patient, this procedure may last between 3 and 6 hours. It does not appear that a rebound overshoot in antibody levels occurs after plasma exchange, but rapid re-equilibration of IgG occurs because 55% of IgG is extravascular [83, 84]. A reduction of 70–85% can be obtained with four to six exchanges in 14 days [3] so we often use a course of six plasma exchanges over 2 weeks, possibly adding one or two more procedures if the patient seems to be deteriorating. Treatment of some patients such as those with TTP or acute Guillian–Barr disease might involve plasma exchange daily for several days.

A model [85] that takes into account the size of the exchange relative to the patient's blood volume, the amount of material available to exchange, the amount of material in both the intravascular and extravascular compartments, the mobility of the material between the pools, and the production and catabolic rate of the material showed good agreement with in vivo observations in patients with hyperbilirubinemia and hypercholesterolemia.

Replacement solutions

Managing the fluid balance of the patient during the exchange may be difficult, especially if the patient has compromised cardiovascular function or is hemodynamically unstable due to the clinical situation. Therefore, it is usually desirable to replace the plasma being removed with an equal volume

of material with oncotic pressure similar to plasma. The most common of these solutions is 5% normal serum albumin. In some situations, it is not necessary to replace the plasma removed with an equal volume of albumin. Instead, some saline (crystalloid) can be used, but this should be no more than 25–30% of the replacement volume and must depend on the patient's specific condition. If the patient has an elevated protein level or is quite stable from a cardiovascular standpoint, some mixture of saline and albumin can be used. On the other hand, if the patient is hemodynamically unstable, it is best to maintain the intravascular volume unless a decrease or increase is indicated to correct the instability.

Using albumin alone or albumin plus saline avoids the possibility of disease transmission and allergic reactions from fresh frozen plasma. The loss of coagulation or other proteins is not so extreme that replacement with fresh frozen plasma is necessary in patients who have a normal coagulation system (Table 19.5). Fresh frozen plasma should be used only when there is a need to replace coagulation factors or other unknown but

Table 19.5 Comparison of changes induced by plasma exchange of 1.0–1.5 plasma volumes with equal volume replacement.

Albumin replacement	FFP replacement
Hematology	
↓ Platelets (30–50%)	↓ Platelets (30–50%)
↑ Granulocytes (2000–3000/mL)	↑ Granulocytes (2000–3000/mL)
↓ Hemoglobin (10–15%)	No change in hemoglobin
Proteins	
↓ Pathological antibodies (60–75%)	↓ Pathological antibodies (60–75%)
↓ All other proteins (60–75%)	All other proteins change to approximate levels present in FFP
Long-term effects depend upon TER, FCR, and S (see the text)	
Coagulation	
↓ Individual factors (60–75%)	All factors approximate levels in FFP
Transient coagulopathy (24–48 hr)	
Electrolytes	
Slight ↓ potassium	↓ Potassium (0.7 mEq/L)
Albumin: Ø bicarbonate (6 mEq/L)	↑ Bicarbonate (3 mEq/L)
↑ Chloride (4 mEq/L)	↓ Chloride (6 mEq/L)
PPF: ↑ bicarbonate	
↓ Chloride	
Citrate and calcium	
Slight ↑ citrate (0.2 mM/L)	↑ citrate (1.1 mM/L)
↓ Total calcium (1.4 mg/dL)	Slight ↓ total calcium (0.3 mg/dL)
↓ Ionized calcium (0.5 mEq/L)	↓ Ionized calcium (0.6 mEq/L)

Source: Chopek M, McCullough J. Protein and biochemical changes during plasma exchange. In: Therapeutic Hemopheresis. Arlington, VA: American Association of Blood Banks, 1980:13.
FCR, Fractional catabolic rate; FFP, fresh frozen plasma; PPF, plasma protein fraction; S, synthesis; TER, transcapillary escape rate.

essential constituents, as in the therapy of TTP. An example of the relative amount of replacement solutions is available from Canada, where data are obtained on a national scale about replacement solutions. Albumin is probably used for about 70% of exchanges [5] Hydroxyethyl starch can be used instead of albumin [86] although this has not gained wide acceptance.

Biochemical changes following plasma exchange

Removal of such a large volume of plasma has several biochemical effects (Table 19.5) [87–89]. Because some platelets are in the plasma being removed, there is about a 30% decrease in the platelet count, which takes about 3 days to return to baseline [90]. The changes in the proteins IgG, IgM, IgA, factor V, ferritin, transferrin, lactic dehydrogenase, serum glutamic oxaloacetic transaminase, and alkaline phosphatase follow closely the decrease expected based on the volume of plasma removed [87, 88]. When no fresh frozen plasma is used for replacement, coagulation test results are quite abnormal at the end of the plasma exchange. For instance, the prothrombin time is usually 20 seconds or more, the partial thromboplastin time is more than 180 seconds, and the fibrinogen is decreased by about 70% [87, 91]. These test values return to baseline in about 24 hours, except fibrinogen, which normalizes in 72 hours [87, 88] (Figure 19.2).

Complement components C3, C4, and CH50 can be depleted when albumin is used as the replacement fluid. However, because of its rapid rate of synthesis, complement is not depleted unless plasma exchange is done daily for several days [92].

There are no clinically important changes in electrolytes as a result of plasma exchange (Table 19.5). Because citrate is usually used as the anticoagulant, and it exerts its effect by binding calcium, an important consideration is the ionized calcium level. If fresh frozen plasma is used for replacement, this provides additional citrate. Citrate toxicity is the result of the hypocalcemia, not the citrate itself. The hypocalcemia may cause symptoms of paresthesia, muscle cramping, tremors, shivering, lightheadedness, and anxiety; when more severe, it can cause grand mal seizures, tetany, and most dangerous of all, electrocardiographic abnormalities (Figure 19.3). Very low ionized calcium levels may cause abnormal coagulation tests, but hemorrhage is not a result of hypocalcemia because severe cardiac arrhythmias occur first. Studies of citrate and calcium metabolism during normal-donor plateletpheresis have established that symptoms only begin to occur when the rate of citrate infusion exceeds 60 mg/kg/hour [93–95]. Although there is some reduction in ionized calcium levels even when albumin is used as the replacement solution [87, 96], the citrate infusion rates are below 60 mg/kg/hour and only approach this rate when fresh frozen plasma is used and the flow rates are substantial. Thus, supplementation with calcium during plasma exchange should be based on each patient's situation [97].

The differences between albumin and fresh frozen plasma as replacement solutions are summarized in Table 19.5.
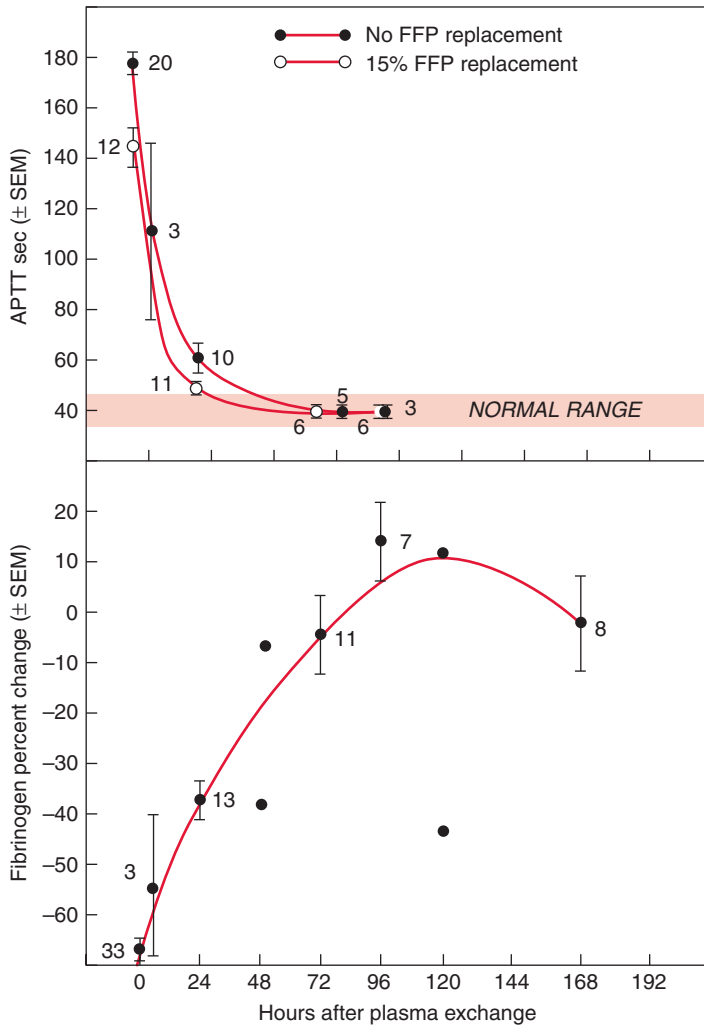
Figure 19.2 Normalization of fibrinogen level and activated partial thromboplastin time after plasma exchange using albumin for replacement. (Reproduced with permission from Chopek M, McCullough J. Therapeutic plasma exchange. Lab Med 1981; 12:745–753. © 1981 American Society for Clinical Pathology and © Laboratory Medicine.)

Complications of plasma exchange

Apheresis of normal donors for the production of blood components is well tolerated with few side effects and only very rare serious complications. However, therapeutic apheresis is carried out in ill patients who should not be expected to react the same as a healthy donor [98]. Deaths have occurred due to therapeutic apheresis, and the mortality rate is estimated to be 3 per 10,000 procedures [99, 100]. Most deaths have resulted from cardiac or respiratory arrest, but deaths due to anaphylaxis,
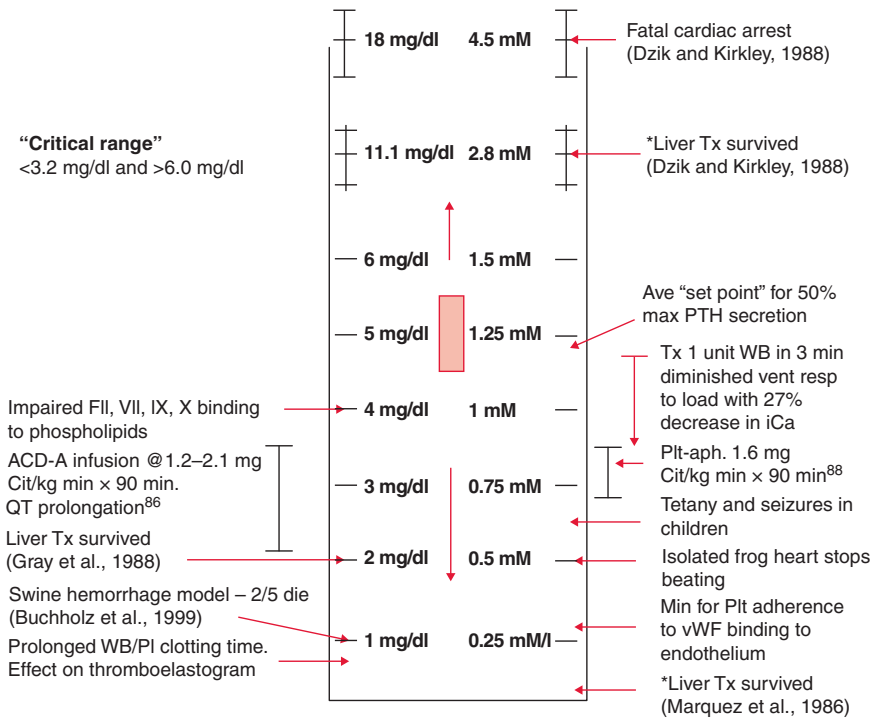
Figure 19.3 Relationship of clinical signs and symptoms to ionized calcium concentration. (Kindly provided by R.J. Bowman, MD. Please refer to references [157–160].)

pulmonary embolus, and vascular perforation have also been reported (Table 19.6) [99, 100]. The nature and incidence of complications will depend somewhat on the condition of the patient prior to plasma exchange. In one comprehensive report of complications of plasma exchange, side effects occurred during 12% of procedures and involved 40% of patients [101]. The incidence of severe complications was 0.5% of procedures. A national registry of therapeutic apheresis procedures in the former East Germany reported complications in 22% of 1945 procedures in 419 patients [102]. There were severe complications in 2%, including cardiac arrhythmia, bronchospasm, adult respiratory distress syndrome (ARDS), and thromboembolic problems. Of the 419 patients, 87 died; of these, 64 died of their underlying disease and 19 of related causes not thought to be due to the plasma exchange. Twelve patients died during or immediately after the plasma exchange, and four of these fatalities were thought to be due to the apheresis procedure [102]. Two of these fatalities resulted from ARDS, one from myocardial infarction, and one from pulmonary embolus. The complication rate was almost twice as great when fresh frozen plasma was used as the replacement solution compared to albumin, although the nature of the complications was not described. The Canadian Apheresis Study Group reported that adverse reactions

Table 19.6 Complications of plasma exchange.

Vascular Access
   Sepsis
   Pneumothorax
   Sternocleidomastoid hematoma
   Air embolus
Hemorrhage from ruptured artery
   Replacement Solutions
   Allergic reactions
   Fever
   Hemolysis
   Hypocalcemia
   Coagulopathy
   Protein depletion
   Transfusion-related acute lung injury (TRALI)
   Anaphylactoid reaction
Procedure
   Fluid imbalance
   Hypotension (ACE inhibitor)
   Anemia
   Instrument malfunction

occurred in about 9% of 58,000 procedures [4]. About two-thirds of these were mild, and only 8% were severe, resulting in a severe reaction rate of 7 per 1000 procedures [4].

In general, plasma exchange is reasonably safe if it is used in appropriate clinical circumstances where there is a rational expectation of benefit to the patient and in which proper nursing care is available for the patient. The complications of plasma exchange can be categorized as those related to (a) vascular access, (b) replacement solutions, or (c) the procedure itself.

Vascular access

The most severe complications of plasma exchange are sometimes related to the vascular access devices [79]. In one study of 391 procedures in 63 patients, the only four severe complications were bacterial sepsis due to an infected catheter, a sternocleidomastoid hematoma, a pneumothorax, and a hemopneumothorax, all resulting from placement of a subclavian catheter. An additional patient exsanguinated from an artery that was lacerated during placement of a subclavian catheter. In another study of vascular access in patients undergoing TPE, there were three severe catheter-related complications in 23 patients who received 28 venous catheters [80]. The complications were a pneumothorax during placement of the catheter, acute respiratory failure due to an air embolus after removal of a catheter, and bacteremia from an infected catheter. Thus, the necessity of using venous catheters is an important part of the risks of plasma exchange.

Replacement solutions

Complications related to replacement solutions include citrate-induced hypocalcemia, coagulation factor depletion, depletion of other functional proteins, electrolyte abnormalities, and transfusion reactions or disease transmission when plasma is used. Allergic reactions such as urticaria or mild fevers are rather common even when albumin is the replacement solution. Progenic reactions can occur to specific lots of albumin [103]. An unusual case of hemolysis due to infusion of hypotonic replacement solution has been reported [104]. The 25% albumin was diluted to 5% in sterile water rather than saline, resulting in the hypotonic solution. Depletion of coagulation factors leading to a bleeding diathesis should not occur because this can be prevented by using fresh frozen plasma as the replacement solution. The same is true for other functional proteins and electrolytes. If fresh frozen plasma is used, febrile or allergic reactions are more common because of the proteins in the plasma. Antibodies in the plasma may cause transfusion-related acute lung injury (TRALI) [105] (see Chapter 14), and ABO-incompatible plasma can cause hemolysis.

Apheresis procedure

Mild reactions are rather common during plasma exchange; they are usually chills (possibly due to infusion of room-temperature replacement solutions) or lightheadedness (possibly due to a vasovagal reaction). These can also be symptoms of hypocalcemia due to citrate infusion, and so when the symptoms begin, the operator often slows the blood flow rate and the symptoms subside. Another procedure-related complication is hypotension or hypertension due to fluid imbalance. Hypotension can be caused by hypovolemia resulting from the blood required to fill the extracorporeal circuit. This can be a substantial volume if the Haemonetics equipment is used because plasma is continually removed until the bowl is filled with red cells. Thus, the volume of the circuit in relation to the patient's blood volume must be considered, and for smaller patients, it may be desirable to prime the circuit with albumin. If this is done, the dilutional effect of the priming solution must also be considered. Hypertension can occur if the volume of fluid returned exceeds that removed. Thus, it is important that the operator closely monitor the fluid balance during the procedure.

Anaphylactoid reactions consisting of flushing, hypotension, bradycardia, and dyspnea have occurred in patients taking angiotensin-converting enzyme (ACE) inhibitors for hypertension [106–108]. Bradykinin (BK) causes vasodilitation and smooth muscle contraction in some tissues. ACE is the major peptidase that inactivates BK [108]. Patients receiving ACE inhibitor drugs have less ability to inactivate BK. Thus situations that promote BK release may lead to hypotensive reactions in patients taking ACE inhibitors. This is thought to occur during therapeutic apheresis, possibly due to contact between the patient's blood is exposed to foreign surfaces of plastic bags, tubing, centrifuge systems, and blood filters. Discontinuation of the ACE inhibitor for 24–48 hours before therapeutic apheresis prevents these reactions [108].

Red cell depletion

Although red cell depletion would not be expected because plasma is removed during plasma exchange, anemia does develop after multiple exchanges [109]. This is probably due to blood remaining in the extracorporeal system, blood removed for laboratory testing, and insufficient red cell regeneration by the patient [109].

19.3 Red cell exchange or erythrocytapheresis

Red cell exchange is done when removal of a large proportion of the patient's circulating red cells is desired. Use of a blood cell separator makes it possible to rapidly remove a patient's red cells and replace them with normal-donor cells while maintaining hemostasis and fluid balance. Red cell exchange transfusion is used to treat or prevent sickle cell crisis [110, 111] (see Chapter 12) and to treat severe malaria [112, 113] or babesiosis [114]. The exchange transfusion procedure is carried out similarly to plasma exchange, except that the red cells instead of the plasma are diverted into a waste bag and donor red cells are recombined with the plasma for return to the patient. A mathematical model can be used to estimate hemoglobin levels after exchange and to project the timing of the next exchange [115].

19.4 Therapeutic cytapheresis

In some patients with hematologic proliferative disorders, symptoms and severe complications can occur because of the high levels of circulating cells. Blood cell separators can be used to treat this by removing abnormal accumulations of leukocytes, platelets, or red cells (Table 19.4). In therapeutic cytapheresis, the blood cell separator instrument is operated similarly to collection of the particular blood component from a normal donor. The procedure is usually lengthened to process more blood and thus remove a large number of cells. The number of cells removed depends on the initial level but can be 10^{11} or even 10^{12}. The peripheral leukocyte or platelet count may be reduced by 20–80% depending on the initial level [116]. Several instruments are available for therapeutic cytapheresis [117] (Table 19.5).

 As in TPE, very few controlled trials of therapeutic cytapheresis have been carried out. There is no doubt that cytapheresis can be used to rapidly lower the elevated cell levels in acute or chronic leukemia and thrombocytosis. Sometimes the procedure is done to prevent potential problems such as central nervous system vascular slugging in patients with chronic myelogenous leukemia, reduction of metabolic problems due to cell lysis during chemotherapy of acute myelogenous leukemia, or prevention of thrombotic episodes in patients with thrombocytosis. In other situations, cytapheresis is done to attempt to treat existing problems such as central nervous system symptoms in acute or chronic myelogenous

leukemia patients with high leukocyte counts. The medical literature is helpful in establishing the general indications for these procedures, but it is often difficult to decide for specific patients whether cytapheresis is necessary and, if done, whether it was beneficial. The following is a summary of the value of therapeutic cytapheresis in specific situations.

Myelogenous leukemias

Symptomatic vascular slugging due to high levels of circulating leukemia cells can be a medical emergency. In one study, leukemic cellular aggregates were the cause of death in 24% of patients with acute myelogenous leukemia and 60% of patients with chronic myelogenous leukemia [118]. All patients who died with leukocyte counts greater than 200,000/mL and half of those who died with leukocyte counts between 50,000 and 200,000/mL had prominent cellular aggregates and thrombi in their tissues. Central nervous system leukostasis can lead to cerebral vascular neurosis with intracranial hemorrhage or thrombosis [119], pulmonary insufficiency [120], or coronary artery occlusion [121]. Patients with acute myelogenous leukemia and blast counts above 100,000/mL are likely to experience acute respiratory distress or fatal intracranial hemorrhage. Contact between adhesion receptors in leukemic and endothelial cells [122] may contribute to the risk of leukostasis. Thus, emergency therapeutic leukapheresis may be used in conjunction with chemotherapy when the blast count is greater than 50,000/mL or approaches 100,000/mL.

   Patients with acute myelogenous leukemia who present with leukocyte counts greater than 100,000/mL (usually mostly myeloblasts) may have serious problems in addition to leukostasis during the first few days of chemotherapy. These include hyperuricemia, renal failure, and disseminated intravascular coagulopathy and are thought to be partly due to the lysis of a large number of leukemia cells. These problems may be reduced or prevented by lowering the leukocyte count by cytapheresis. Usually one or two cytapheresis procedures suffice until the initial chemotherapy takes effect.

   In patients with chronic myelogenous leukemia, symptoms of leukostasis may develop when the leukocyte count exceeds 100,000/mL. If the clinical condition warrants, one or two cytapheresis procedures can be used to rapidly lower the leukocyte level. Chemotherapy should also be started because cytapheresis is not effective maintenance therapy.

Chronic lymphocytic leukemia

It is not clear that high leukocyte levels in these patients create problems. Although the count can be lowered rapidly by cytapheresis, this is not recommended unless the patient has symptoms due to high levels of circulating lymphocytes.

Thrombocytosis

Thrombocytosis that occurs as part of myeloproliferative diseases can lead to hemorrhage or thrombosis, especially in the central nervous system.

Platelet counts of 1,000,000/mL or more may occur. Cytapheresis can be used to lower the platelet count rapidly [116, 123, 124] with the expectation that complications of thrombocytosis will be prevented.

Complications of cytapheresis

In general, there are fewer complications from cytapheresis than from plasma exchange because in cytapheresis the small volume of cells removed is replaced by saline and/or anticoagulant. However, patients undergoing therapeutic cytapheresis are often quite ill, and the cytapheresis procedure may be risky because of other factors such as bleeding or central nervous system problems. Reaction rates of 8–10% have been reported for therapeutic plateletpheresis and 14–21% for therapeutic leukapheresis [117].

19.5 Photopheresis

Photopheresis (photochemotherapy or extracorporeal photochemotherapy) is a form of adoptive immunotherapy (see also Chapter 18) involving the combination of photochemotherapy and leukapheresis. In photopheresis, the patient is given a dose of 8-methoxypsoralen, and 1–2 hours later leukapheresis is performed. A mononuclear cell concentrate is collected containing approximately $1–2 \times 10^{10}$ cells, or about 10–15% of the patient's total circulating lymphocytes. After the mononuclear cell concentrate has been collected, it is removed from the instrument, heparinized, and diluted with the patient's plasma and saline. The mononuclear cell concentrate is then passed for about 2–3 hours through a disposable clear plastic plate that rests between two banks of ultraviolet A lights. The ultraviolet irradiation activates the psoralen to become an alkylating agent, but this is only temporary because the effect only persists while the cells are exposed to the ultraviolet light. The ultraviolet-treated mononuclear cell concentrate is then transfused to the patient as a form of adoptive immunotherapy. It is important that the mononuclear cell concentrate has a low hematocrit because red cells absorb the light and thus interfere with the effect of the ultraviolet light on the mononuclear cells.

There are several possible mechanisms of action of photopheresis, but in general it is believed that the process leads to a cell-mediated immune response. This may then eliminate or reduce expanded disease-producing clones. Suggested possible mechanisms of action of photopheresis include (a) induction or suppressor or cytotoxic T cells; (b) stimulation of the development of clone-specific suppressor cells; (c) increase in natural killer (NK) cells; (d) decrease in circulating dendritic cells, or (e) activation of monocytes with release of cytokines, including interleukins 1 and 6 and tumor necrosis factor; (f) presentation of intact but inactivated cells to the immune system, enabling development of an immune response to the cells; and (g) induction of apoptosis with presentation of processed tumor antigen to primed dendritic cells resulting in an antitumor response. It

Table 19.7 Therapeutic applications of photopheresis.

Malignancy	Cutaneous T-cell lymphoma
	Sézary syndrome
Transplantation	Cardiac allograft rejection
	Lung allograft rejection
	Renal allograft rejection
	Acute and chronic graft-versus-host disease
	Prevention of graft-versus -host disease
Autoimmune	Progressive systemic sclerosis
	Systemic lupus erythematosus
	Rheumatoid arthritis
	Psoriatic arthritis
	Pemphigus vulgaris
Other	Coronary restenosis
	AIDS-related complex

Source: Sniecinski, I. Photopheresis. In: McLeod BC, Price TH, Drew, MJ, eds. Apheresis: Principles and Practice, 2nd ed. Bethesda, MD: AABB Press, 1997, pp. 521–535.

seems likely that more than one mechanism of action occurs because photopheresis appears to be beneficial in several clinical situations.

Photopheresis has been used in several clinical situations [1, 125, 126] (Tables 19.4 and 19.7). The only disease in which this is accepted therapy is for cutaneous T-cell lymphoma when at the advanced stage [127, 128]. Early stages of cutaneous T-cell lymphoma have survival rates of 8 years, and this has not been improved by photopheresis. However, for patients with advanced disease, a response rate of 75% was reported and the median survival was extended from about 30 months to well beyond 60 months [129]. These results applied to patients with both Sézary syndrome and mycosis fungoides, probably as a result of lymphocyte apoptosis [130]. The skin disease improves and the leukocyte CD4, CD8, and Sézary cell counts decrease [130], usually into the normal range. Photopheresis is given as a course of treatment. Usually two treatments are given on consecutive days each month for 6–12 months. After patients have stabilized, the interval between treatments is lengthened. Some patients develop fever following transfusion of their mononuclear cells and the erythrodermia usually becomes more severe, although this diminishes as the treatments progress.

Photopheresis has been used or is under investigation in the treatment of other autoimmune diseases such as pemphigus vulgaris, scleroderma, rheumatoid arthritis, and systemic lupus erythematosus, but its benefit has not yet been established in any of these conditions.

Considerable interest has developed in the use of photopheresis in transplantation. Photopheresis has reversed rejection of transplanted hearts [131–133], kidneys [134], and lungs [135]. Some centers have incorporated photopheresis into routine posttransplant immunosuppression, usually involving two treatments monthly as an

empirical regimen, but the role of photopheresis in managing or preventing rejection of transplanted organs is not clear.

Photopheresis is used as an adjunct in the management of acute or chronic graft-versus-host disease following hematopoietic cell transplantation [136–140]. This may be due to an effect on cytotoxic CD8+ T cells and on dendritic and NK cells [136]. Data about the value of ECP in the treatment of graft-versus-host disease (GVHD) is mostly observational data from case reports or small, uncontrolled studies. The only substantial control trial was a multicenter prospectively randomized study comparing ECP plus standard therapy with standard therapy alone [141]. There was a significant improvement provided by ECP and it also appeared to have a steroid sparing effect. Therefore, the aggregate of data suggests that ECP is probably helpful in therapy of chronic GVHD but data is inconclusive regarding its role in acute GVHD.

19.6 Therapeutic apheresis using selective adsorption columns

Plasma exchange may remove as much as 150 grams of plasma protein to extract only 1–2 grams of pathogenic protein while removing other important proteins such as immunoglobulins and coagulation factors [142]. Selective adsorption of the offending material is a more appealing approach, but unfortunately it has not gained wide use.

Familial hypercholesterolemia

Apheresis can be used to treat familial hypercholesterolemia. The general approaches involve (a) immunoadsorbent affinity columns using antilipoprotein B, (b) chemical adsorbent affinity columns using dextran sulfate, (c) chemical precipitation with heparin, and (d) secondary filtration [143]. In the procedures, the patient's blood is passed through a blood cell separator and the plasma fraction is passed over the columns in a continuous flow. About 3–4 L of plasma is usually treated during 2 hours. The patient is usually treated every 2 weeks. Adverse reactions, which are most commonly chills and/or hypotension, are mild and occur in fewer than 5% of procedures [144]. These methods can produce a decrease in low density lipoproteins (LDL) of 130–170 mg/dL or 44–81% [145, 146]. This treatment is associated with prevention of the progression of carotid or aortotibial vascular disease [144]. There is no doubt that extracorporeal therapy using LDL-specific apheresis is safe and efficacious [147], but it is very costly and involves a substantial commitment by the patient.

Autoimmune disease treated with dextran sulfate columns

Dextran sulfate columns such as those used to treat hypercholesterolemia have also been used for selective immunoadsorption of anti-DNA from the

blood of patients with systemic lupus erythematosus [148, 149]. The clinical value of this therapy is not established.

Immune disease treated with staphylococcal protein A columns

SPA binds IgG—especially IgG classes 1, 2, and 4—and also immune complexes. SPA cannot be infused because of severe toxicity, but plasma exposed to SPA bonded to silica beads, sepharose, or polyacrylamide can be transfused to patients with few side effects. The SPA procedure can be carried out in two ways. Approximately 500 mL of blood is withdrawn as for an ordinary blood donation, then the plasma is separated and passed over the column in the laboratory. The treated plasma is then transfused to the patient. Alternatively, a larger volume of plasma (approximately 1000–2000 mL) can be processed through the SPA column by placing it in the plasma line of a blood cell separator. The plasma is recombined with red cells and returned to the patient continuously throughout the procedure. Any blood cell separator used for plasma exchange can be used for SPA column processing.

Early enthusiasm for the SPA column in malignancy was not substantiated but interest developed in using the columns in patients with autoimmune disease [150]. When the autoimmune nature of idiopathic thrombocytopenia was established, SPA columns were used to treat refractory ITP by removing the antiplatelet IgG antibody [151–153]. Patients underwent six treatments over about 2 weeks. SPA treatment was very effective, providing an increase in platelet count in 46–66% of patients refractory to other therapies [152, 153]. SPA columns were used to treat inhibitors (IgG antibodies) to coagulation factors VIII and IX in hemophilia A and B [56, 154] until activated coagulation factor concentrates became available to bypass the inhibited portion of the coagulation system. SPA columns have also been used for removal of IgG antibodies in patients who are alloimmunized and refractory to platelet transfusions [155], patients with myasthenia graves [20], hemolytic-uremic syndrome, rejection of transplanted organs, rheumatoid arthritis [156], and HIV-related thrombocytopenia [157]. In most of these situations, occasional individual patients seem to benefit from the therapy, but many do not. The continued production of antibody and/or the presence of the antigen makes treatment by physical removal of IgG antibody of limited effectiveness.

Although several reports contended that the side effects of the SPA columns were modest, some side effects have been severe and have included generalized pain, fever, rigors, sweating, hypotension, nausea, abdominal pain, dyspnea, cyanosis, disorientation, arthralgia, skin rash, vasculitis, purpura, thrombosis, renal failure, urticaria edema, and chest pain [158]. Some of these effects may result from the SPA-caused binding of immune complexes liberating free antibody, complement activation, and increased helper T-cell activity [158].

While the concept of specific removal of IgG is appealing, the SPA column is not available in the United States and is not much used worldwide.

Therapeutic apheresis in children

TPE or cytapheresis can be performed on even very small children [159–162]. The major consideration is that the instruments are designed for adults and thus the extracorporeal volume may be too large for small patients. This can be overcome by priming the instrument with red cells, albumin, or other combinations of fluids. The blood flow rates through the instrument are not high for adults but may represent a large portion of a small child's blood volume. Therefore, problems can arise quickly if there are difficulties with the lines or blood flow. Also, the rate of return of blood and solutions can be much greater in relation to the total blood volume of a small child, and thus citrate or other complications can occur more frequently than in adults, if adjustments in blood flow are not made to reflect the small patient's blood volume [161–163].

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20 Quality Programs in Blood Banking and Transfusion Medicine

The public's concern with the safety of the blood supply and the recognition of the extent of errors in the provision of medical care [1] have highlighted the importance of quality in blood banking and transfusion medicine [2]. Medical and scientific developments will greatly increase the complexity of blood center operations and hospital transfusion service will also necessitate sophisticated quality assurance and process control programs and processes.

20.1 Quality systems

The AIDS epidemic created a new regulatory climate [3–6]. Blood products are now produced in a manner more similar to the production of pharmaceuticals than was true in the past when blood centers operated more like hospital clinical laboratories [4, 5, 7, 8]. The manufacturing environment also led to changes in the organizational philosophy and the introduction of new concepts of quality and process control [4, 8, 9].

Quality programs and systems within transfusion medicine take two different general forms. One involves blood component production and the blood supply system and the other involves the patient transfusion system. The approach used to ensure the highest quality of the blood components in the blood supply system is similar to that used in the manufacture of pharmaceuticals, whereas the approach used to ensure that patients receive the highest quality transfusion therapy incorporates some of this but also is part of a hospital's overall quality-of-care program.

One important measure of quality is the safety of the blood supply. This is described in more detail in Chapter 15. Several strategies have been used to accomplish this improved safety, but the purpose of this chapter is to describe specific activities targeted toward improving the quality of the blood operation and of transfusion practice.

Quality and quality assurance systems

In contemporary manufacturing practices, quality is usually the most important standard. Not only is it valuable in itself, but it also contributes

Table 20.1 Differences between blood component production and pharmaceutical manufacturing.

	Blood Bank	Pharmaceutical
Source material	Human	Chemical
	Biologic	Nonbiologic
	Heterogeneous	Homogeneous
Source information	Human memory	Assays
	Human interaction	Assays
Source of supply	Limited	Extensive
Critical information available	After manufacturing	Before manufacturing
Product variability	Substantial	Minimal
Usable life	Short	Long
Laboratory tests	Medium/low precision	High precision
	Biologic	Chemical
Regulatory impact	Donors	No donors
	Manufacturers	Manufacturers
	Supply affected	Supply unaffected
Lot size	Small	Large
Final check before use	Crossmatch	None

Source: Reproduced from McCullough, J. The continuing evolution of the nation's blood supply system. Am J Clin Path 1996; 105:689–695.

to productivity by eliminating wastage, redundancy, and the costs of troubleshooting failed products, and maintaining customer loyalty. In the detection model to achieve quality in a manufactured product, a random sample of the final product is tested for certain desirable and undesirable characteristics [8]. However, this selective testing accepts that some defective products will be produced, move through the system, and be distributed. Theoretically, the customer finds the defective product and returns it to the manufacturer. "Reliance on inspection as a mechanism of quality control was discredited long ago in industry"[10]. In the case of transfusion therapy and blood components, the detection approach means that some patients may receive a product of less than desirable quality or safety, since there is usually no effective way to detect a "poor-quality" component before it is transfused. Other reasons that the detection approach is not ideal for blood components is that the "raw material" or donated blood is variable (Table 20.1) and cannot be standardized like chemicals in a manufacturing process [4, 8, 9]; and since each donation becomes a "lot," it is impossible to carry out quality control testing on a random sample of material from each lot [8].

A more contemporary approach to quality is the prevention model [10]. This model assumes that errors will occur and attempts to minimize them by rigidly controlling the process [8, 9]. It also changes the paradigm from error detection to error prevention. This approach is especially suited for blood and components. While this discussion and terminology may seem like jargon or concepts applicable only to traditional assembly-line

Table 20.2 The quality hierarchy.

Stage	Activities performed
Total quality management	Management approach aimed at long-term success through customer satisfaction
Quality assurance	Includes stages below the "costs of quality"
Quality system	Comprehensive efforts to meet quality objectives fulfills requirements for quality
Quality control	Operational techniques to measure quality outputs from process

Source: Nevalainen DE. The quality systems approach. Arch Pathol Lab Med 1999; 123:566–568.

manufacturing, these principles can be extremely valuable in the production of blood components. The US Food and Drug Administration (FDA) requires demonstration of the safety, potency, purity, and efficacy of licensed blood components. For the patient and the practicing physician, it is essential to be as certain as possible that the component is exactly as desired and expected. Thus, the effect of the transfusion can be attributed to the product itself. If the patient has the expected beneficial effect, this is a good outcome. If the component has the expected and desired characteristics but the expected effect does not occur, this can be attributed to patient-related factors. Then the next steps in diagnosis or therapy can be taken because the lack of benefit is not the result of a poor-quality component or unknown adventitious agents in the component. In addition, adverse effects of the transfusion can be more easily investigated if the component is assuredly standard and meets all expected criteria for quality. Three terms that reflect different aspects of the quality program are: quality management, quality assurance, and quality control [11]. Quality management refers to the role of the organization's leadership in creating a quality organization and running from suppliers through the organization and beyond to customers.

The quality assurance system is ". . . the sum of the activities planned and performed to provide confidence that all systems and their elements that influence the quality of the product are working as expected" [7]. The goals of the quality assurance system (program) are to decrease errors, obtain credible results consistently, improve product safety, and improve product quality [7]. The quality program should provide predictable, high-quality, and cost-effective collection, processing, and distribution of

Table 20.3 The elements of total process control.

Process maps
Standard operating procedures (SOPs)
Performance-based training
Competency assessment
Records

Table 20.4 Process analysis and management.

Define the work output
Understand the process
Develop a prototype process
Validate the process
Train to comply with process SOPs
Assess competency
Implement the process

blood and components [9]. To achieve this, "all parts of an organization work together to create the cultural environment and infrastructure necessary to support the program" [9].

Quality control testing has been a routine part of blood center and hospital transfusion services activities for years. Some hospital blood banks and transfusion services developed innovative programs that were very effective [12]. However, for the most part, quality control was limited to specific testing to identify "defective" products. Thus, the tests and methods are selected to provide feedback on the state of a process or product. Contemporary quality systems begin with a clean, strong commitment to quality from the top leadership of the organization and incorporate the concepts of quality assurance and quality control (Table 20.2) [13].

There are two general approaches to ensuring quality: control of the process and control of the product. It is presumed that good control of the process provides better control of the final products and leads to a relatively standardized product. In other types of manufacturing this control is achieved using the five elements of "total process control" (Table 20.3). In this approach, the process map identifies the critical steps of the process, the standard operating procedures (SOPs) define the procedures to be used, the training is based on the SOPs, and assessment is used to determine that employees understand and can carry out the SOPs [8,9]. A similar approach to process control is "process analysis and management" (PAM), a seven steps program (Table 20.4). These involve educating participants about PAM, developing a new or revised process

Table 20.5 Quality principles according to the AABB-.

Organization and leadership
Facilities, work environment, safety
Human resources
Customer focus
Supplies and materials management
Equipment management
Documents and records
Information management
Management of nonconforming events
Monitoring and assessment
Process improvement

Table 20.6 Good manufacturing practices.

Code of Federal Regulations	Zuck modification [a]
General	
Organization and personnel	Personnel management
Buildings and facilities	Facilities
Equipment	Equipment
Production and process controls	Process controls
Finished product control	Labeling
Laboratory controls	Quality control and auditing
Records and reports	Record keeping
	Calibration
	Error management
	SOPs

[a]Zuck TF. Current good manufacturing practices. Transfusion 1995; 35:955–966.

including new SOPs, validating the process, training the staff to carry out the process, determining whether staff members are trained satisfactorily and how this will be monitored, and finally implementing the new process. The practical application of quality principles of the American Association of Blood Bank (AABB) involves 12 elements (Table 20.5). Additional structures that provide for a quality operation are the FDA's Good Manufacturing Practices (GMPs) (Table 20.6) and the International Standards Organization's system (Table 20.7).

Table 20.7 International Organization for Standardization 9000 Quality System Elements.

Element
Management responsibility
Quality system
Contract review
Design control
Document and data control
Purchasing
Customer-supplied product
Product identification and traceability
Process control
Inspection and testing
Control of inspection, measuring, and test equipment
Inspection and test results
Control of nonconforming products
Corrective and preventive action
Handling, storage, packaging preservation, and delivery
Control of quality records
Internal quality audits
Training
Servicing
Statistical metrics

20.2 Quality assurance in the blood supply system

Good manufacturing practices

The FDA has over the years defined in the Code of Federal Regulations (CFR) their expectations of process control. These are called GMPs (Table 20.6). Zuck describes these in a more expanded form (Table 20.6) based on the common requirements to comply with the CFR [8]. Each of the parts or components in the GMPs is applied to each blood center operation, whether it be blood collection, infectious disease testing, or the production of components. The following discussion uses the Zuck [8] structure, but the content can be applied to other quality systems.

Personnel

The director of the blood program activity should have the authority necessary to ensure the overall quality of the operation. The qualifications of personnel carrying out processing should be defined. There should be a written training program that should include training in concepts of GMPs as well as training in the specific processing and testing methods. There should be a written description of the competency required before an individual can begin working on actual production for patient use. Thereafter, the individual's continued competence should be demonstrated through an ongoing program of monitoring or proficiency testing. Capabilities of personnel should be commensurate with their responsibilities.

Facilities

The principles governing the facility operation are that production areas should be separate from other activities such as research or testing; access to the production area should be limited; equipment used for production should not be shared with nonproduction activities; there should be procedures for responding to spills or accidents; and environmental testing should demonstrate adequate control of the area used for processing [7]. An environmental testing program for air quality and pest control should be in place to establish the quality of air and the absence of rodents or other pests. There should be adequate space so that overcrowding leading to errors is avoided. Space should be adequate to allow proper storage of materials and quarantine of products not ready for patient use. In addition, wall coverings, floors, and countertops should be such that adequate cleanliness can be maintained and that there are smooth surfaces designed for aseptic processing. The flow of people, incoming materials, and material in processing should be organized to avoid or minimize the likelihood of microbial contamination, introduction of adventitious agents, or cross-contamination among cells from different individuals. Security of the area should be such that unauthorized personnel are excluded.

Equipment

Equipment should be selected so as to be optimally effective for its desired function and to minimize the likelihood of microbial contamination, introduction of adventitious agents, or cross-contamination of cells among

different individuals. Initially, all equipment should be calibrated, making sure that calibration is done in the critical operating range of the instrument. There should be an ongoing program of calibration to ensure that the equipment remains in a satisfactory performance range. The equipment must be maintained in a clean state.

All supplies and reagents should be recorded and should be stored under proper conditions. Many blood centers have a materials management group that logs in all supplies and reagents, stores them, and monitors their release into different operations areas.

Production and process controls

Total process control is the concept that quality, safety, and effectiveness are built into the product [8, 9]. Each step in the process must be controlled to meet quality standards. The expectation is that this approach provides a predictable outcome or product from the process. This results in a final product with a minimum of variability and as close as practical to the desired product every time it is produced. Two important parts of total process control are the use of SOPs and critical control points.

Standard operating procedures

All procedures should be carried out using written instructions or SOPs. First, it is essential to determine where SOPs are necessary, then who will develop them. These procedures should be developed in a systematic way, use a standard format, and be readily available to the staff. All steps should be carried out exactly as specified in the SOPs. There should be a specific policy and procedure for changing procedures (change control), including definition of personnel authorized to make changes. A permanent record of all outdated SOPs should be maintained so that in the future there can be no doubt as to how each specimen was processed.

Critical control points

A separate part of process control is the use of "critical control points." This involves defining the critical steps in each method, where clinically important errors might occur and establishing systems or check steps to minimize the likelihood of an error occurring or being undetected.

Other important parts of process control are control of changes in processes, validation of processes, the quality control testing program, and traceability.

Laboratory quality controls

Laboratory controls must be used to ensure that reagents are working properly and that the blood components have the expected composition. The tests for reagents are not usually specified in the CFR but are established locally as part of each institution's quality program. Testing of the components or final product is specified in the CFR. This defines the test to be done, the proportion or frequency of testing, and acceptable limits of results.

Labeling

There should be written definition of the information to be contained on the label of each container of cells intended for patient therapy. This prevents mix-ups of components and provides information regarding the proper handling of the cellular product. The material to be specified on the label is defined in the GMPs.

Records management

There should be written description of the records to be maintained and the length of retention. Records retained should at least make it possible to determine the source (donor) of the cells, donor-related information, all manipulations to which the blood or components were subjected, and key quality control tests related to the safety, purity, and potency of the product. The records should make it possible to trace all steps clearly; they should be indelible; and they should identify the person who performed the work. Record keeping should be performed concurrently with the work; it is unacceptable to complete records after the work is completed.

Computers

The FDA considers that computer software is a device [14]. The FDA has taken this position because computers and their related software now play a key role in the control of the operation of the blood center and thus are major contributors to the safety of the blood. However, this position by the FDA has major implications. It means that the software must be developed, tested, validated, and maintained in a manner similar to the development of a medical device. Computer software then must go through the process of becoming licensed by the FDA under device licensing procedures. While this process has improved the quality of computer software, it adds to the cost and time necessary to develop these systems [14].

Internal quality audits

For the manufacture of biologics and blood, a program of internal audits should be carried out. Quality audits should be focused on systems, not on specific procedures. For instance, in addition to counseling a staff member who makes an error, the factors that made the error possible should be identified to determine whether corrective action is indicated. Examples of systems to consider are: (a) donor suitability and blood collection; (b) incoming material testing, quarantine, and release; (c) cell processing or testing procedures; (d) storage and shipping; (e) staffing and personnel competency; (f) reagents and materials management; (g) quality control testing and outcome actions; (h) equipment maintenance and calibration; and (i) error, accident, and adverse reaction reporting systems.

Management of nonconforming events

A more extensive description of errors in blood banking and transfusion medicine is given later in this chapter. Nonconfirming events were previously called the error and accident detection and management system part of GMPs. The program should be written and appropriate portions

should have SOPs and be part of the laboratory's SOP system [15]. Nonconforming events that occur during cell processing or testing should be documented and investigated to determine whether this indicates any shortcoming in the processing method. In addition to any action in response to a particular event, there should be periodic review of these events to determine whether systemic or process changes are indicated. Thus, there should be a system of corrective action that will define the actions to be taken as a result of nonconforming events or adverse reactions.

Some errors or nonconforming events may reveal that affected blood products have been distributed for patient use. This activates a product recall or withdrawal [16]. These are complex but rather rigid processes defined by the FDA.

Adverse reaction files

A record of adverse reactions to the transfusion of blood or components should be maintained. The adverse reactions should be reviewed at the time they are reported and also periodically to determine whether they suggest that any shortcomings exist in the processing methods or that modifications of methods or procedures should be made.

Organization and quality assurance personnel

In the quality assurance program recommended by the FDA [7], the personnel responsible for the program report separately from those responsible for the "manufacturing" operation. In this way, the operation is separate from the quality activity and those responsible for quality have equal authority over the production activities. The principle of separation of authority for quality from production is key, as it establishes that the quality function is equally as important as the production function.

International Organization for Standardization

The International Organization for Standardization (ISO) 9000 quality management system is used worldwide in a variety of settings including health care [13]. The ISO 9000 system involves 20 elements (Table 20.7) that are quite similar to the elements of GMPs and cover the same activities. Some blood centers are ISO certified [17]. This is a complex process, but ISO 9000 certification implies a high level of quality that is recognized worldwide.

American Association of Blood Banks Quality program

The AABB has developed a quality program, the modules of which are listed in Table 20.5 [18]. The program contains the elements of total process control and GMPs. It begins with general organizational issues such as the statement of the goals of the program and the organizational structure, in which there is independent reporting of the quality unit to top management, separate from operations personnel. Human resource elements include position descriptions, evidence of training, and systems to ensure that adequate training is provided and that competency is

monitored. The validation element includes procedures for validation, calibration, and preventive maintenance of equipment and records of this. Supplier qualification is an important concept. Specifications or product requirements are provided to suppliers, and there is a system to determine that suppliers meet these specifications continually. In process management, critical control points are defined, SOPs written, and controls put in place to monitor specified conditions for each critical step. The documentation program includes the format for records, lists of pertinent documents, and the change process. Label control involves written procedures for handling labels, preventing mix-ups, revising labels, and discarding obsolete labels. Procedures for the identification of all errors, adverse events, or accidents are an essential part of a quality program. This process is used to identify shortcomings in the operation and make improvements. An internal audit or assessment program is also valuable; when carried out by staff members who do not directly perform the procedures it provides another way of identifying potential shortcomings so that corrective action can be initiated. All of this program is based on the concept of continuous improvement, which involves constant vigilance for shortcomings in the operation, collection of baseline data, development of solutions to improve the operation, implementing the solutions, and monitoring the impact. This is a very thorough program that can be the basis of a quality operation.

Other blood bank quality systems

Another way of thinking about quality programs divides the activities into four categories: [1] processes, [2] people, [3] material and equipment, and [4] management [9]. This involves thinking about what is being done, who is doing it, the tools they use to carry out their tasks, and the overall systems and leadership of the program. In the program, flow charts are used to demonstrate the process, policies are developed defining the expected outcomes at different steps, and SOPs are written to provide step-by-step instructions to carry out the process. Within the process there may be some particularly important points that determine the outcome. These are established as "critical control points," and systems are established to check the process at these points. Before putting any process into operation, it is necessary to "validate" it to ensure that the process in fact produces the intended result. Equally important as a sound, clearly defined process is the people who carry it out. Important steps in the acquisition of a competent staff are selection, training, work assignment, validation of competency, and maintenance of skills and competency [19]. The necessity that materials and equipment function as expected has been mentioned above in the description of quality programs for blood production. This applies equally to the hospital blood bank operation. It is essential that reagents have the potency and accuracy expected and that equipment functions as expected so that test results will be valid. In order for a quality program to be effective, it is necessary that the leaders of the organization be committed to this. It will not be possible to develop and implement a program without the support and leadership of top

management. In addition, for a successful ongoing quality program, there must be a commitment from the organization's leaders to search for continuous improvements. Some of the activities that provide this are internal audits of the processes, people, materials, and equipment; the follow-up on adverse events or errors; and the commitment to maintaining a knowledgeable staff.

Berte [20] has categorized the tools for improving quality in a transfusion service as those dealing with quality control, quality assurance, and quality systems. Examples of quality control are equipment maintenance, calibration, and reagent testing. Quality assurance tools are the use of quality indicators, or teams, and tools for systems include vendor certification, corrective action plans, and internal audits. These and many other activities she describes are nice examples of a rather comprehensive quality program. Another example of a practical approach to the use of this type of quality assurance program is described by Galel and Richards [21]. In addition, computers can be used to provide better structure for data capture and thus facilitate monitoring quality [22].

20.3 Errors in transfusion medicine

Many errors occur during medical care [1], and most medical injuries are the result of error, not negligence [23]. Analyses of errors in transfusion medicine and the development of systems that could be used to reduce these errors and, thus, improve quality and safety is an important part of transfusion therapy. The incidence of errors in blood banking and transfusion medicine is difficult to establish. It may be unrealistic to achieve an error rate less than 1/10,000 in repetitive tasks [12]. Errors may lead to serious complications such as the transfusion of infectious or ABO incompatible units. Fortunately, most blood bank transfusion medicine errors do not harm patients [24]. Fatal hemolytic transfusion reactions almost always (92%) are due to an error [25]. Errors related to fatalities most frequently involve giving the unit of blood to the wrong patients [25,26]. In an analysis of errors that led to transfusion of blood to the wrong patients, 58% occurred outside of the blood bank and 25% in the blood bank [24,27,28]. Examples of the kinds of errors are: failure to identify the patient, phlebotomy error, blood issued for incorrect patients and not detected at the bedside, or incorrect sample used for testing [24,27]. Laboratory testing error accounted for only 7% of errors leading to transfusion of the wrong patient. In a separate study, 7% of wristbands were found to contain some error [29], which could lead to disastrous results in transfusion therapy.

Concern about errors stimulated a review of error detection and management in other industries such as aviation or nuclear power as they might be applied to blood banking and transfusion medicine. From this review, a transfusion medicine error system has been developed termed Medical Event Reporting System for Transfusion Medicine (MERS-TM) [30–32]. This system can be used as part of existing quality systems and involves detection, description, classification of errors, and a database

allowing analysis and a systems approach to quality improvement [12]. Foss and Moore [12] have described a similar error management system involving error definition, detection, analysis, management, and prevention. An important factor is the (un)willingness of staff to report errors, but this does not seem to be a problem in blood banking and transfusion medicine [33].

In the management of errors, it is important to address immediate problem-solving but also to have a system to track and trend errors in order to conduct a root cause analysis when indicated [34].

20.4 Quality assurance in the transfusion service

Quality as it relates to the transfusion service can be considered as quality of the (a) blood and components, (b) blood bank operations, (c) transfusion procedure, and (d) transfusion practice. The blood and components must be as safe as possible, provide the expected potency, be readily available as needed, and be stored and handled properly within the hospital; the patient testing must be carried out in a timely and accurate manner; and the blood and components must be transfused to patients safely and effectively. The therapeutic uses of blood and components should reflect good medical practice and the record systems should be adequate to document all of the activities mentioned here. Quality concepts and emphasis should be part of every segment of an organization [35]. An effective comprehensive approach to this is the Quality School developed at the Mayo Clinic.

The descriptions of quality programs in the previous sections of this chapter were directed more to organizations that collect blood and prepare components. Some of the issues and critical control points are the same for a blood production organization and a hospital transfusion service, but some are different. The AABB quality program and ISO 9000 related to a manufacturing process were difficult to apply to the transfusion service. Thus, the quality system essentials were developed [36] as a more simplified version that can be applied to the hospital transfusion service. Not surprisingly these ten essentials are similar to GMPs and ISO 9000 elements. However, they are worded and explained in a way that puts them in the context of a hospital transfusion service.

Another important aspect of a quality transfusion service is the availability of a realistic disaster plan.

Blood supply

The quality of the transfusion service begins with ensuring an adequate supply of safe and high-quality blood components. Some hospital blood banks produce a portion of their supply themselves, while many purchase their blood and components from a community blood center. If the hospital produces some of its supply, then the above description of quality in programs for the blood supply will apply to that activity. However, even if the hospital does not produce its supply, the transfusion service professionals have a responsibility to ensure the adequacy and quality of

Table 20.8 Activities in the transfusion of blood and components that are part of the transfusion medicine quality program.

Release of the blood components from the blood bank
Transportation of the blood components from the blood bank to the patient care area
Identification of the unit and the patient prior to initiating the transfusion
Selection of the proper intravenous route and vascular access device
Administration of solutions used during transfusion of the blood component
Operation of devices used during the transfusion
Nursing care before, during, and after the transfusion
Documentation of all steps in the process and the nursing care information

the components that make up the hospital's inventory. This involves close interaction with the supplier to determine that a suitable quality program exists and that the blood and components being provided contain the expected potency and meet the safety expectations of the hospital. This becomes a form of "vendor qualification" similar to that used for the purchase of other supplies.

Hospital blood bank operations

There are several types of activities that should be considered part of the quality program in the operation of the hospital blood bank (Table 20.8). The first of these begins with the collection and labeling of the patient specimen and steps to ensure the quality of the specimen (see also Chapters 13 and 14). One of the most common errors in clinical laboratories is mislabeling of specimens. This can cause a fatal hemolytic transfusion reaction due to ABO incompatibility, and so it is an important part of a quality program designed to minimize these specimen-labeling errors. Of course it seems obvious that it is essential to carry out the correct tests in each situation for each specimen, but errors do occur and it is important to ensure that the proper test is ordered, that the records reflect this, and that the laboratory staff actually perform the test requested. It is expected that all tests will be done correctly according to the laboratory SOPs and that all test results will be reported timely and accurately. Complete, accurate, and legible records of all steps must be maintained. An adequate supply of safe, high-quality blood components has already been mentioned as an important part of the quality of the operation of the hospital blood bank and transfusion service. The blood and components must be stored under conditions that maintain optimum quality and effectiveness at the time of transfusion, and the procedures for release of blood from the blood bank must be sufficiently detailed to minimize the likelihood that blood will be dispensed to the wrong patient.

Transfusion of blood and components

Most of the activities in the transfusion of blood and components occur outside of the blood bank and are not carried out by blood bank personnel (Chapter 13). Thus, the quality assurance program for these activities is a joint one usually involving both the blood bank and the nursing service. It

Table 20.9 Activities of a hospital blood bank subject to quality programs.

Specimen collection, labeling, and quality
Carrying out the correct tests in each situation for each specimen
Carrying out all tests correctly according to the laboratory SOPs
Reporting all test results timely and accurately
Maintaining complete, accurate, and legible records
Acquiring an adequate supply of safe, high-quality blood components
Storing the blood components under conditions that maintain optimum quality and effectiveness at the time of transfusion
Providing blood components requested for patient care timely and accurately

is essential that the blood bank and transfusion medicine staff be involved in the program because they have special knowledge and an approach that adds to the quality program. The particular activities in the transfusion process that lend themselves to inclusion in the quality program are described in Table 20.9.

Ensuring that the blood components are transfused correctly so as to provide the maximum effectiveness while minimizing the chance for an adverse effect is another important aspect of quality in the transfusion service. This begins with release of the blood components from the blood bank and continues with the transport of the blood components from the blood bank to the patient care area. Systems should be in place to ensure that the proper unit is released from the blood bank. Although there will be subsequent checks of the identity of the unit and the patient, this is one of the redundant steps to minimize the likelihood that a patient will receive the incorrect unit. Administration of the incorrect unit, leading to ABO incompatibility and a hemolytic transfusion reaction, is the most common cause of transfusion-related fatality. Thus, redundancies are built into the system to prevent these errors. Activities that are important parts of assuring the quality of the transfusion are discussed extensively in Chapter 13. These include: transporting the unit, handling the unit at the patient care area, venipuncture and vascular access, infusion solutions, nursing care of the patient, infusion devices, blood warmers, and blood filters (Table 20.8).

An essential part of all of these activities is thorough, legible documentation. This is an invaluable part of the quality program, as it provides the record of exactly what is done and how it is done. This is the basis for the continuous review to maintain quality and also is the basis for problem solving and corrective action when this is necessary.

20.5 Quality assurance in patient therapy

Quality in transfusion therapy
Increased requirements by accrediting agencies, more medical knowledge, the increasing use of practice guidelines, improved methods of managing

some patient situations, the availability of drugs that avoid transfusion, the public and patient concerns about blood safety, and cost containment efforts are all interacting to strengthen the need for quality improvement in transfusion practice.

There is general evidence indicating that the medical use of blood and components is not ideal. Consensus development conferences for the use of platelets, albumin, and fresh frozen plasma have all concluded that some inappropriate use occurs and have provided recommendations for more suitable indications (see Chapters 11 and 12). One study of blood use in coronary artery bypass surgery at 18 different hospitals found a 10-fold difference in the number of red cells and a 40-fold difference in the total number of components transfused and that 43% of these transfusions were unnecessary [37]. Conversely, undertransfusion might represent another form of inappropriate transfusion therapy [38]. Several approaches have been taken to continually improve transfusion practice. The major approach is continuing education of physicians. This is done in a wide variety of settings such as general and specialty medical meetings, medical societies, hospital medical staff meetings, publications in the medical literature, audio tapes, teleconferences, and written materials provided by blood suppliers [39]. In addition, the use of blood within the hospital has been monitored in an effort to determine the quality of transfusion practice and form the basis of changing physicians' behavior. These several approaches are described in more detail in the following.

Transfusion committee

This committee of the medical staff is required by the Joint Commission, formerly the Joint Commission on Accreditation of Healthcare Organizations (JCAHO). The committee may be chaired by the blood bank medical director, but it is preferable if another member of the medical staff holds this responsibility. This broadens the involvement of the medical staff and may increase the likelihood of acceptance of the committee's actions. The transfusion committee is a key part of the overall quality program for the hospital transfusion service. The committee should be aware of and possibly approve the blood supplier and thus can influence the quality and availability of the blood supply. In addition, the committee should have some kind of system to monitor blood utilization using the data and reports prepared by the blood bank staff. The committee also would monitor adverse events, errors, and accidents and approve the blood bank's overall quality plan. The committee would be aware of the results of inspections and accreditation findings and use its influence to ensure that the blood bank has the resources needed to carry out the responsibilities. Thus, the transfusion committee can be a major force in the quality of the blood bank and transfusion service operation.

Medical indications

The indications for transfusion are changing (see Chapters 11 and 12), resulting in decreased blood utilization and thus improved safety for patients. In addition, the changes in donor selection and testing and in the

organization of blood banks has reduced the infectivity of blood and made the blood supply safer than ever (see Chapter 15). All of these approaches are resulting in improvements in the overall quality of transfusion practice. As blood transfusion has become more directly linked to specific indications, its use has decreased, with an attendant decrease in the complications of transfusion. Changed indications have been developed in response to patients' concerns about blood safety and also to reduce the costs of patient care by avoiding unnecessary therapies. However, at the same time, medical and scientific knowledge is accumulating that forms the basis for altering the indications for some blood components. Examples of this are improvements in the management of acute blood loss and anemia, thus lowering the hemoglobin indication for routine transfusion; the use of erythropoietin to avoid red cell transfusions; and reduction in the platelet count indication for prophylactic platelet transfusion (Chapter 11). Another factor that plays into the changing indications is the increased use of practice guidelines in health care [40]. As practice guidelines are developed for diseases or treatments involving transfusion, indications for transfusion are included, thus bringing some standardization and consistency to transfusion practice. For some situations, practice guidelines specifically related to transfusion are used [41]. The indications for blood and component use are implemented in several ways [39]. Transfusion medicine professionals provide education through medical staff meetings or other medical education forums. Lectures by transfusion medicine professionals can improve compliance with the transfusion guidelines [42], but the durability of the improvement is not known. In general, educational programs are necessary and provide some benefit but by themselves do not accomplish the extent of change desired. Comprehensive programs are more effective [39].

Transfusion audit

The Joint Commission requires that the medical staff audit the appropriateness of all transfusions. Usually this is done through the transfusion committee. The transfusion audit relates to the transfusion therapy guidelines established by the medical staff. In the transfusion audit, the criteria for the use of the different blood components established by the medical staff are compared with the use of blood components. All or a selected number of transfusions are reviewed to determine whether the use of the blood components is consistent with the medical staff guidelines. Transfusion audit can become a complex and time-consuming activity because, depending on the criteria, a rather extensive amount of information may be needed about each transfusion to determine whether it meets the indications specified for that component. In addition, such a review is by necessity retrospective. Thus, the transfusion has already occurred and the information is used to attempt to alter physicians' behavior in the future but not to prevent what might be an inappropriate therapy at the time. In the early experience with transfusion audits, some improvement was shown in the reduction of the number of transfusions that did not conform to hospital guidelines. Examples were the decline in

the number of single-unit transfusions, considered at the time to be inappropriate. Some believe that the gains that can be obtained by this retrospective audit method have been achieved, and the process may no longer be contributing to further improvements [43, 44], but others have found the audit process to be valuable [45, 46].

Blood ordering practices

Another approach to improving both the quality of transfusion practice and the operational efficiency of the blood bank was the establishment of standard or maximum blood orders for common surgical procedures. This makes it possible for the surgeon to know the amount of blood that will always be available for particular types of operations and enables the blood bank to avoid the excess inventory and blood wastage that results from setting aside excessive amounts of blood for elective surgical procedures [47–50]. This program, which is described in more detail in Chapter 10, has been very effective in reducing blood wastage and improving blood availability for patients undergoing elective surgery. The standardization of blood ordering practices becomes part of medical care guidelines and the transfusion audit program and is then an important part of the quality program in transfusion therapy [51].

20.6 Summary

Quality may seem obvious and simple, but it is complex to integrate into everything from donor recruitment and medical assessment to transfusing a patient. This chapter is an attempt to provide a general concept of quality as it relates to blood products and transfusion therapy and a view of the many applications of quality systems.

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