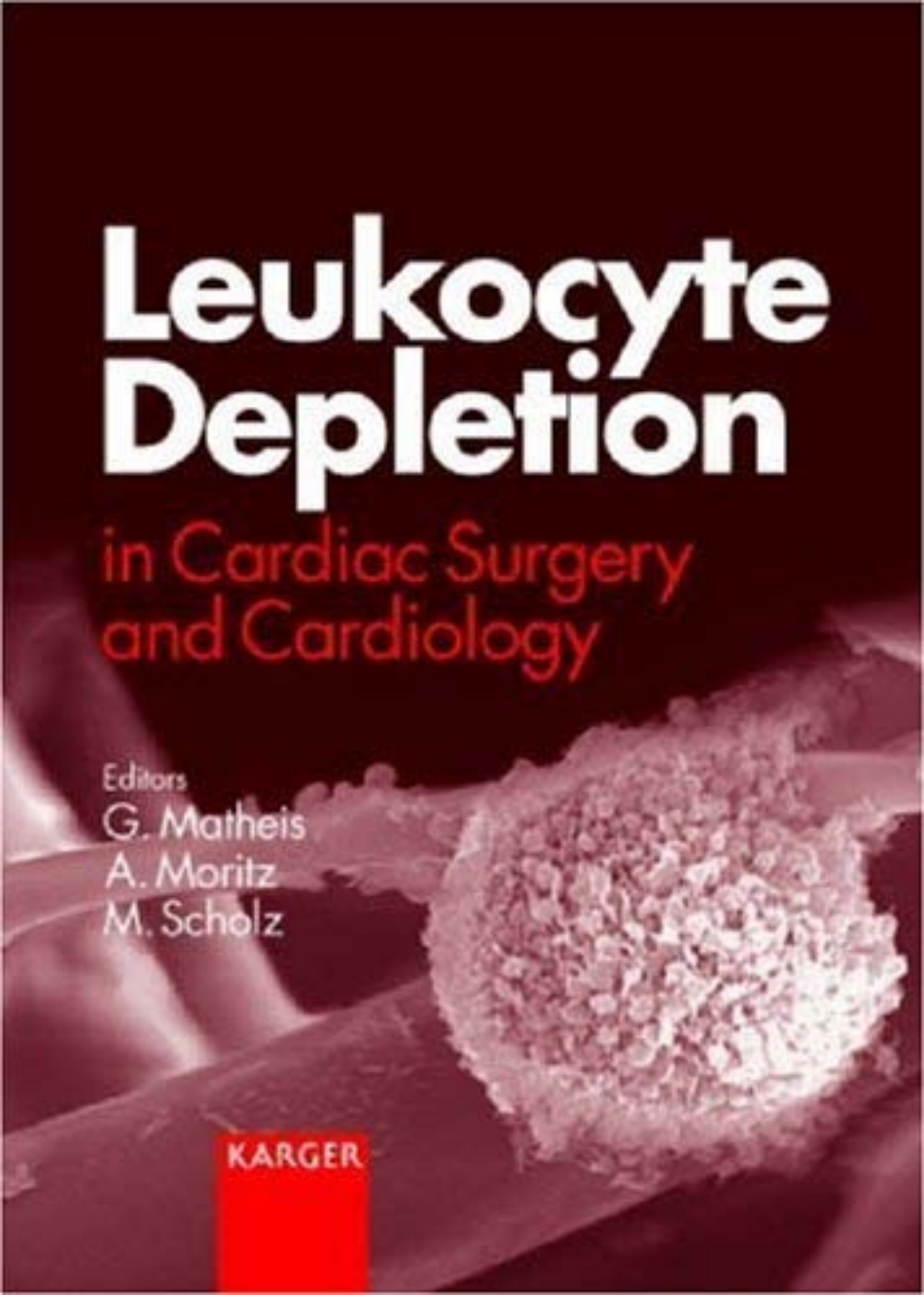
Leukocyte Depletion

in Cardiac Surgery and Cardiology

Editors
G. Matheis
A. Moritz
M. Scholz



KARGER

Leukocyte Depletion in Cardiac Surgery and Cardiology

Editors

Georg Matheis *Frankfurt*
Anton Moritz *Frankfurt*
Martin Scholz *Frankfurt*

32 figures and 15 tables, 2002

KARGER

Basel · Freiburg · Paris · London · New York ·
New Delhi · Bangkok · Singapore · Tokyo · Sydney

Georg Matheis, Dr. med.
Anton Moritz, Prof. Dr. med.
Martin Scholz, Dr. med.
Department of Thoracic and Cardiovascular Surgery
J.W. Goethe University, Frankfurt

www.karger.com
Printed in Switzerland on acid-free paper by Reinhardt Druck, Basel
ISBN 3–8055–7281–6

Contents

Preface

The concept of leukocyte depletion by means of a leukocyte removal filter was introduced by Fleming in 1926 and was also proposed by Wright. During the past decades, leukocyte depletion has become an important tool for the prevention or reduction of the pathogenic effects (posttransfusion syndrome, virus infection/transmission) of blood transfusion. The reduced pathogenicity of leukocyte-depleted blood products significantly reduced the overall cost per patient transfused. Currently, an increasing number of countries recommend or make universal filtration of blood transfusions mandatory. While the filtration of all blood products is not yet mandatory in the US, the medical community, following the recommendations of the Blood Products Advisory Committee of the FDA, has been advising that all donor blood should be filtered to remove white cells. The American Red Cross has publicly stated its intention to reach this goal by the end of 2001. Routine filtration of all whole blood, red blood cells and platelets has become mandatory in Germany as of October 2001.

In cardiac surgery, where transfusion of blood products or perfusion is required, leukocyte depletion has evolved as an important tool. Patients receiving foreign leukocytes have to deal with the posttransfusion syndrome, transient immunosuppression, allogeneic immune reactions, and virus infections or (re)activation. Those who undergo cardiac surgery with cardiopulmonary bypass (CPB) and do not even receive foreign blood products may develop systemic inflammatory response and organ dysfunction mainly due to the activation of neutrophils.

The first specific leukocyte-depleting arterial line filter for CPB was introduced in 1991 and has subsequently been evaluated by several investigators (see

the chapter by Palanzo, Allentown, Pa.). To date, several investigators have reported on the beneficial effects of leukocyte depletion in cardiac surgery regarding both clinical effects and cost reduction.

Despite the favorable results of leukocyte depletion in cardiac surgery with CPB, the exact mechanistic definition of the beneficial impact of leukocyte filters in limiting the pathogenicity of CPB remains quite elusive. To further advance leukocyte filter technology and filtration strategies, it is important to understand the complex biology of activated neutrophils and their role in cardiac surgery with or without CPB.

Neutrophil effector mechanisms represent major immune responses of the innate immune system. Independent of antigen presentation pathways via histocompatibility complexes I and II, neutrophils are activated after binding of their Fc receptors to immune complexes or of their complement receptors to central complement factors, such as C3a, C5a, C3b. Following activation, opsonized pathogens are eliminated via phagocytosis or exocytosis by lysosomal enzymes (e.g. muraminidase, myeloperoxidase, elastase) and oxygen radicals, mechanisms that may contribute to immunopathogenesis.

It is well established that CPB and the operation per se activate neutrophils via numerous pathogenic factors, such as contact with artificial surfaces of the extracorporeal circuit, and that activated neutrophils elicit severe endothelial injury by overshooting effector mechanisms. Neutrophil adhesion to and/or transmigration through the vascular endothelium are triggered by surface molecules, such as selectins and integrins, that allow firm adhesion to endothelial adhesion molecules. Complex intercellular mechanisms account for the subsequent neutrophil transendothelial migration, which involves disruption of interendothelial cell contacts (e.g. cadherins), enzymatic digestion of the extracellular matrices and consecutive formation of transient or persistent edema.

In the first chapter of this book, the biology of neutrophils and their pathogenic effects are considered by Gourlay et al. (London). This chapter demonstrates that leukocyte depletion is a logical strategy to limit neutrophil-mediated disorders in cardiac surgery. The cerebral sequelae of cardiac surgery with CPB are described by Scholz et al. (Frankfurt). The following chapter by Sheppard (Southampton) presents mechanisms and technical aspects of leukocyte depletion to limit the pathogenicity of activated leukocytes.

As the neutrophil-related pathomechanisms differ depending on the intervention (transfusion, transplantation, extracorporeal circulation, reperfusion), the strategies of leukocyte filtration have to be adapted to the particular clinical setting and will be discussed separately. The subsequent chapters cover transfusion (Henschler, Frankfurt) and transplantation (Scholz and Matheis, Frankfurt). The chapters on perfusion are introduced by a systematic study of filtration modalities (Matheis and Scholz, Frankfurt), followed by an introduction to

reperfusion injury during CPB by Krishnadasan et al. (Seattle, Wash.). Gu et al. (Groningen), Palanzo (Allentown, Pa.) and Matheis et al. (Frankfurt) report clinical studies with different objectives in adults. Allen analyzes some of the few studies available on CPB and leukocyte filters in infants. The chapter by Martin (Freiburg) deals with leukocyte filtration of blood cardioplegia. In the last chapter, Berg et al. (Frankfurt) speculate about future filtration strategies, such as bioactive cytokine filters.

In contrast with transfusion medicine, leukocyte filtration in cardiac surgery has not yet become a standard procedure despite the broad documentation of its beneficial effects. This may change when current studies on leukocyte filtration in cardiac surgery become available to a broader audience of practising surgeons and perfusionists.

This book reviews the experience with experimental and clinical leukocyte filtration, summarizes the state of the art in clinical application, and provides an outlook on the possible future role of leukocyte depletion in cardiology and cardiac surgery.

Georg Matheis
Anton Moritz
Martin Scholz

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 1–12

Leukocyte Biology and Pathogenicity in Cardiac Surgery and Cardiology: The Need for Leukocyte Depletion

T. Gourlay, G. Asimakopoulos, K.M. Taylor

Department of Cardiac Surgery, NHLI, Imperial College Medical School,
London, UK

Patients undergoing cardiopulmonary bypass (CPB) involved in the repair of cardiac lesions are known to exhibit an inflammatory response mediated by the activation of white cells. The inflammatory response may be systemic, and is often described as the systemic inflammatory response syndrome (SIRS). In extreme cases, SIRS may be life threatening [1]. The mechanisms underlying this unwanted side-effect of CPB are many and complex; however, the involvement of leukocytes has been well established. A number of therapies have evolved, aimed at reducing the extent of the inflammatory response, and included in these is the recent introduction of leukocyte-depleting filters for use in perfusion circuits. The effectiveness and implications of this technology, when applied in specific areas of the perfusion circuit, will be discussed in detail in later chapters in this book. Similar leukocyte depletion techniques have recently been applied in other branches of medical practice, in interventional cardiology, the treatment of SIRS/sepsis, and filtration of malignant cells, amongst others. The use of leukocyte depletion during invasive cardiological procedures is an interesting and fast-expanding branch to those concerned with the related local inflammatory response and the ischaemia/reperfusion-associated inflammatory mechanisms resulting from circulatory interruption [2].

Inflammation

In general terms, inflammation is the non-specific reaction of vascularised tissue to a variety of injurious stimuli. The inflammatory process involves the

generation and activation of a complex network consisting of both molecular and cellular systems. Our knowledge of the mechanisms that regulate inflammation has improved significantly in recent years. The leukocyte, regarded as a major participant in the battle against micro-organisms, may also be responsible for some of the adverse effects associated with systemic inflammation.

The adult differential leukocyte count is attained at puberty and ranges from 4 to 10×10^9/l. The leukocyte population consist of neutrophils (40–75%), lymphocytes (20–50%), monocytes (2–10%), eosinophils (1–6%) and basophils (<1%). All of these are involved to some degree in the inflammatory processes, but in this chapter we will discuss only those known to play a significant role relevant to cardiac surgery and cardiology.

Neutrophils: Function and Kinetics

Specific cytokines, the colony-stimulating factors, direct bone marrow progenitor cells to produce the three granulocyte subtypes: neutrophils, eosinophils or basophils. Eosinophils and basophils are produced and eliminated at a much lower rate than neutrophils. Their mode of activation and their role in acute inflammation suggest that they are of limited relevance to this chapter and, therefore, they will not be discussed further.

After exiting the bone marrow, neutrophils become protagonists of host defence with a half-life of 7–10 h whilst in circulation. Their activation and mobilisation towards injured tissue or areas of bacterial invasion aim to protect the host and comprise critical steps in inflammation. Neutrophils are drawn to inflammatory sites by specific chemotactic factors. Stimulation of neutrophil receptors by these factors leads to neutrophil movement via modification of intracellular proteins.

Pivotal steps resulting in the generation of chemotactic factors are the release of bacterial polysaccharides, complement activation, plasmin activation and factor XII (Hageman factor)-related activation of the clotting system and of the kinin-kallikrein system. Certain chemotactic agents are produced by a variety of activated leukocytes, macrophages and endothelial cells. Components of the complement cascade system and interleukin-8 (IL-8) are some of the most potent neutrophil chemoattractants [3].

In addition to chemotaxis, the binding of inflammatory cytokines to their neutrophil surface receptors triggers off enzymatic reactions leading to the so-called ‘respiratory burst’, placing the neutrophil in an activated state. Activated neutrophils interact intensively with endothelial cells in an adhesion cascade that precedes the entrance of the neutrophils into tissue. The initial step in the

process of neutrophil extravasation and migration into subendothelial tissue is loose 'rolling' of the neutrophil to vascular endothelium under hydrodynamic shear flow (see Krishnadasan et al. [4] in this book). Rolling is mediated by glycoproteins named selectins, the primary sequence of which was first described in 1989 [5]. The selectins comprise a group of three molecules that are closely related in structure and function: L-selectin, E-selectin and P-selectin. L-selectin is expressed on most leukocytes while E-selectin is expressed on endothelium and P-selectin on endothelium and platelets. Rolling of activated neutrophils onto their endothelial ligands often progresses to firm adhesion. This step is regulated by surface receptors called integrins.

Integrins comprise the largest group of adhesion receptors and are found on most cell types, including leukocytes. They are transmembrane cell surface proteins that respond to signals of cellular activation and mediate their function by binding to specific ligands. Each integrin contains a non-covalently associated α- and β-chain with characteristic structure. Integrins are classified into subgroups based on the β-chain. The most widely expressed integrins belong to the β_1-class and are also known as very late antigens (VLA). For a student of the acute systemic inflammatory response, however, the β_2-integrins are of higher significance. They are expressed exclusively on the surface of leukocytes and can be upregulated within minutes to hours. The integrin $\alpha M\beta_2$ (also referred to as Mac-1 or CD11b/CD18), in particular, plays an important role in leukocyte activation and extravasation [6].

Following leukocyte activation and rolling on the endothelium by means of selectin expression, binding of integrins to their endothelial ligands regulates many leukocyte responses including firm adhesion to endothelium, migration into tissues, degranulation and phagocytosis. Once adherent, neutrophils secrete proteases, such as elastase and metalloproteinases, and generate toxic oxygen-derived free radical metabolites contributing to damage to the endothelium. Of these neutrophils, some will project pseudopodia and migrate between and through endothelial cells [7]. Neutrophils that cross the endothelium and enter subendothelial tissues do not return into the circulation. Their survival in the tissue is prolonged during inflammatory processes, under the influence of certain cytokines, allowing them to fight the injuring agent. This highly regulated neutrophil-led defence system of the organism against potentially threatening insults may often be hazardous for the tissues it aims to protect. Despite its strong antibacterial potential, the neutrophil has little ability to distinguish between foreign and host antigens.

After extravasation, toxic products generated by the neutrophils can destroy normal cells and dissolve connective tissue [8]. This 'exaggerated' form of inflammatory response can be local or systemic and can vary from clinically undetectable to fatal.

Neutrophil Activation and Cardiac Surgery

In recent years, there has been increasing interest in the investigation of the, now established, association between cardiac surgery and the systemic inflammatory response. A number of inflammatory insults take place during cardiac surgery; these include contact of humoral and cellular blood components with the artificial material of the CPB circuit, ischaemia/reperfusion phenomena, bacterial endotoxin release and mechanical operative trauma. The relative contribution of these factors to the overall inflammatory picture, however, is still under debate. Dysfunction of major organs associated with cardiac surgery has been well described in the literature, and its origin may be infective or related to atheromatous disease, but may often be exacerbated by inflammatory processes [9]. In this context, neutrophils are thought to be of major significance.

Cardiac surgery causes an initial neutropenia followed by acute mobilisation of neutrophils from the bone marrow and leukocytosis [10]. Simultaneously, neutrophils become activated through complement-related and complement-unrelated mechanisms. Practically all characteristic steps of neutrophil activation, including cytokine secretion, 'respiratory burst', increased adhesion to endothelium and generation of toxic metabolites take place intra- or postoperatively. The timing of CPB-related neutrophil activation is shown for the measurement of plasma neutrophil elastase in figure 1.

Neutrophil activation during CPB is dependent on the action of a variety of inflammatory mediators. Complement activation and generation of the strongly chemotactic complement components C3a and C5a reach peak plasma levels at the end of CPB [11]. Raised plasma levels of the also strong leukocyte chemoattractant IL-8 are detected after CPB [12]. Most plasma IL-8 probably originates from activated endothelium [13].

Currently, the study of patients undergoing cardiac surgery without CPB is providing an insight into the relative importance of CPB as an initiator of inflammatory response. The generation of plasma chemoattractants in patients undergoing cardiac surgery was blunted when no CPB was used [14, 15]. Endothelin-1, a potent activator of neutrophils, is produced during cardiac surgery and was shown in one study to contribute to neutrophil activation and increased adhesiveness to endothelial cells [16]. Cardiac surgery with CPB results in generation of oxygen-derived free radicals by activated neutrophils [17]. Plasma levels of neutrophil elastase, a protease released by neutrophils following their activation and adhesion to endothelium, also increase significantly after cardiac surgery with CPB [18]. The increase is, however, significantly lower when no CPB is used [15].

In addition to immediate 'full-blown' neutrophil activation, neutrophil priming is another potential concept involved in the CPB-related inflammatory

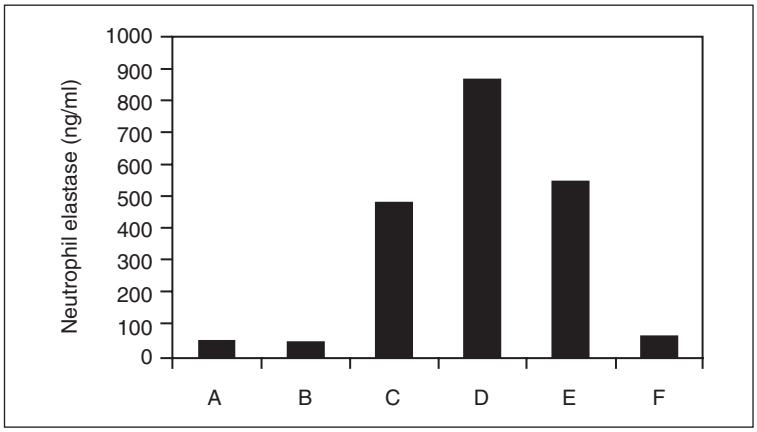


Fig. 1. Typical time kinetics for neutrophil elastase measured by ELISA in patient plasma before, during and after cardiac surgery with CPB. Enhanced elastase levels measured by ELISA do not necessarily reflect the extent of pathogenesis since ELISA detects the inactive form of elastase complexed with protease inhibitors. The ability of the patient plasma to neutralize neutrophil elastase seems to be a more relevant marker to assess the risk for developing leukocyte-mediated disorders. This ability depends in part on the patient's redox status within the tissues because oxygen radicals prevent the formation of inactive elastase-proteinase inhibitor complexes. Thus, neutrophil activation combined with oxidative stress, e.g. during ischaemia/reperfusion is a critical situation during cardiac surgery with CPB [Scholz and Matheis, pers. commun.]. A = Preoperatively; B = before CPB; C = after release of the cross-clamp; D = end of surgery; E = 1 h after surgery; F = 24 h after surgery.

phenomena. This has been described as 'a change in neutrophil status such as the neutrophils show enhanced responsiveness to a second activating stimulus' [19]. Neutrophils become predisposed to systemic inflammatory changes during surgery while a postoperative stimulus, such as tissue ischaemia or infection, leads to their ultimate activation. The risk for tissue damage related to primed neutrophils appears to be highest at approximately 12 h after CPB [19, 20].

In general terms, once activated, neutrophils display increased adhesive capacity, demonstrated through altered expression of surface adhesion molecules. The expression of β_2-integrins has been investigated extensively in patients undergoing cardiac surgery. The integrin $\alpha M\beta_2$ (CD11b/CD18), in particular, is upregulated on the neutrophil surface, reaching its peak expression during CPB and up to 4 h postoperatively. In contrast, the VLAs (β_1-integrins), which bind primarily to components of the extracellular matrix, do not alter their surface expression after cardiac surgery [21, 22].

Neutrophils and CPB-Related Lung Injury

Most major organs exhibit functional impairment after cardiac surgery. Ischaemia related to atherosclerotic changes may often be the primary cause of dysfunction of the myocardium, kidneys and intestine. Systemic inflammatory response, however, is regarded as a major factor exacerbating injury of the lungs and myocardium in particular. Pulmonary dysfunction after CPB was first described in the early years of cardiac surgery and continues to attract considerable interest among clinicians and researchers. Although extreme forms of lung injury, such as adult respiratory distress syndrome (ARDS) are uncommon after modern cardiac surgery, patients often suffer from pulmonary dysfunction postoperatively, as indicated by changes in the alveolar-arterial oxygenation gradient, intrapulmonary shunt, pulmonary oedema, pulmonary compliance and pulmonary vascular resistance [23]. The CPB-associated lung injury is a complicated model of organ dysfunction, where ischaemia-reperfusion injury and endotoxaemia are the primary inflammatory stimuli. After the administration of protamine, the neutrophil count in the pulmonary artery exceeds the count in the systemic arterial blood, suggesting that neutrophils are sequestrated in the lungs [24, 25]. The concentration of neutrophils in bronchial lavage fluids was higher after CPB in comparison to a control group of patients [26]. Histological changes in the lungs of animals confirm that neutrophil sequestration occurs in association with endothelial cell swelling and leakage of erythrocytes into the alveolar space starting mainly after reperfusion [27]. The significance of leukocyte activation in CPB-induced lung injury was demonstrated by the finding that pentoxifyllin, an inhibitor of leukocyte activation, reduces lung dysfunction in cardiac surgical patients [28]. Similarly, leukocyte depletion with an arterial filter was shown to attenuate lung injury after CPB in human and animal studies [29, 30]. Further studies demonstrated that inhibition of matrix metalloproteinases [31] or elastase [32] reduced lung injury in animals undergoing CPB. Furthermore, it is likely that the mechanism of acute lung injury by neutrophils involves an upregulation of $\alpha M\beta_2$ (CD11b/CD18). Indeed, lung dysfunction after CPB has been shown to be reduced when animals were treated with the $\alpha M\beta_2$ inhibitor NPC 15669 [33] or with an anti-CD18 monoclonal antibody [34]. The above findings have established a neutrophil-dependent model for CPB-related lung dysfunction. Activation of neutrophils with upregulation of adhesion molecules, neutrophil adhesion to the endothelium of lung vessels and endothelial damage through proteases appear to be the main steps of the underlying pathophysiological mechanism.

Neutrophil Activation after Myocardial Ischaemia – CPB Related

There is recent evidence suggesting that myocardial ischaemia and the changes in myocardial performance and structure associated with it have a significant inflammatory component. There is little direct evidence, however, linking the activated neutrophil with the transient dysfunction of myocardial segments after CPB. In a recent study, transcardiac veno-arterial differences of IL-6 and of activated neutrophils revealed that there was myocardial inflammatory cytokine production and neutrophil sequestration in the myocardium of patients undergoing coronary artery bypass grafting (CABG) [35]. In an earlier animal trial, piglets subjected to leukocyte depletion during CPB showed better preservation of left ventricular function [36]. In support of these findings, one study showed that postoperative left ventricular wall abnormalities were associated with serum levels of IL-8 and IL-6 [37].

Neutrophil Activation after Coronary Angioplasty

The role of the neutrophil in the pathogenesis of CPB-unrelated myocardial ischaemia and extension of myocardial injury has been established in recent years. Neutrophils have been shown to be involved in the development of myocardial injury during ischaemia and reperfusion. Percutaneous transluminal coronary angioplasty (PTCA) can be regarded as a clinical model of postischaemic inflammation. The intervention leads to the release of inflammatory mediators as a result of endothelial injury. The procedure has been shown to result in neutrophil activation. Although the mechanism of neutrophil activation after coronary angioplasty has not been fully clarified, neutrophil-stimulating agents, generated by the injured endothelium, probably play a crucial role. It has been demonstrated that chemoattractants are released in the early reperfusion period after myocardial infarction and balloon recanalisation. These chemoattractants may act as inflammatory mediators causing neutrophil activation [38]. For instance, CRP levels increased more than sixfold above baseline when measured 48 h after PTCA [39]. Soluble stimuli, released after the procedure, induce neutrophil integrin expression and oxygen free radical production [40]. Further studies have directly demonstrated neutrophil activation in patients after coronary angioplasty. In most cases, the changes were more prominent in coronary sinus blood samples than in peripheral blood, suggesting that the activation process was cardiac in origin.

Neutrophil activation, as measured by CD11b increase or neutrophil elastase release, occurred after PTCA [41, 42]. Interestingly, the changes were

more prominent in patients who developed restenosis after PTCA in one study [43]. Furthermore, coronary angioplasty is associated with enhancement of neutrophil adhesion, aggregation and chemiluminescence [44]. There was also an increase in the percentage of leukocytes with adherent platelets after PTCA [45]. These findings are consistent with the results of studies using animal models of arterial injury. One hour after transluminal injury to the mouse femoral artery, the denuded surface was covered with platelets and neutrophils in one trial [46]. Studies aimed at investigating the potential role of leukocyte depletion therapy in minimizing the inflammatory response in these patients are currently under way, and these studies are showing considerable promise.

Monocytes

Monocytes mature in the bone marrow from promonocytes, enter the circulation and migrate into tissues where they differentiate into macrophages. Macrophages concentrate especially in lung, liver, spleen and lymph nodes. Monocytes and macrophages are attracted and activated through the influence of certain cytokines. Once activated, they secrete further pro-inflammatory cytokines which are capable of activating neutrophils and endothelial cells. Monocytes and macrophages bind IgG and concentrated antigen on the cell surface. They present antigen to lymphocytes and phagocytose the antigen [47]. Monocytes are attracted to sites of inflammation by monocyte chemotactic protein and interact with endothelium as a part of the extravasation process. The three-step adhesion cascade – rolling, firm adhesion, transmigration – described for neutrophils also takes place.

Effects of Cardiac Surgery on Monocyte Activation

The inflammatory insults that induce neutrophil activation during cardiac surgery may also cause activation of monocytes. The time course of monocyte activation and its role in the inflammatory process, however, differ from those associated with neutrophils. In common with neutrophils, monocyte counts tend to decrease during cardiac surgery with CPB [48]. The initial monocytopenia is followed by monocytosis and monocyte activation during the first postoperative hours. Monocyte activation postoperatively can be characterised according to the upregulation of specific surface molecules. It is also associated with the secretion of pro-inflammatory cytokines, such as IL-6 and IL-8, although these are also produced by activated endothelial cells and, therefore,

are not monocyte specific. IL-8 plasma levels increase significantly 2–6 h after surgery and correspond with increase of monocyte chemotactic factor [12, 49].

The expression of the membrane-associated endotoxin receptor CD14 is reduced on peripheral blood monocytes while plasma levels of its soluble form increase significantly 20 h after cardiac surgery with CPB [50]. Tissue factor expression on circulating monocytes, a marker of monocyte activation, was unchanged in the immediate postoperative period but increased significantly 20 h after CPB in another study [51]. Although upregulation of monocyte CD11b was not detected in humans undergoing CPB [22], it occurred during 2–6 h of simulated CPB [52]. Interestingly, blockade of complement activation in simulated CPB reduces expression of monocyte CD11b and formation of monocyte-platelet conjugates [53].

The role of activated monocytes in inflammatory organ injury has been recognised in recent years. In the lung, blood monocytes migrate into the interstitial and alveolar space where they are transformed into macrophages. Macrophages are important for the generation of the acute inflammatory lung injury, a condition frequently associated with cardiac surgery. Alveolar macrophages are activated within minutes of the inflammatory insult in the lung and secrete chemoattractants for neutrophils and monocytes. As a result, larger numbers of neutrophils and monocytes are recruited and adhere to the pulmonary microvascular endothelium. Activated monocytes migrate into lung tissue and exert toxic effects [22]. It is likely that the mechanisms described above also take place after cardiac surgery, although the subject requires further investigation. In one study, surface expression of β_2-integrins on alveolar macrophages increases significantly after cross-clamp release [54].

In this chapter we have described the critical role of leukocytes in the inflammatory responses associated with both open heart surgery and, to some extent, cardiological procedures. The biology and pathogenicity of the leukocyte was the focus of this chapter, but it is important to understand that much remains to be determined in this field, and it is currently the focus of considerable research effort worldwide, encouraged by the availability of new and appropriate research tools. A number of therapies have been investigated and applied in an effort to minimise the deleterious effects of the inflammatory response, amongst others, these include the use of protease inhibitors, the modification of the biomaterial surfaces of the heart-lung machine to enhance biocompatibility, and the use of leukocyte depletion during open heart surgery and other areas of medical and surgical practice. The use of leukocyte depletion has been, and continues to be, extensively investigated with a considerable body of evidence currently supporting its utilisation in a broad spectrum of medical fields, which now extend beyond cardiac surgery and cardiology to SIRS/sepsis amongst others.

References

1 Kirklin JK, Westaby S, Blackstone EH, Kirklin JW, Chenoweth DE, Pacifico AD: Complement and the damaging effects of cardiopulmonary bypass. J Thorac Cardiovasc Surg 1983;86: 845–857.

2 Watanabe S, Iseke H, Tanaka K, Ishibashi H, Tanaka K, Takai Y, Ejiri N: Attenuation of reperfusion phenomenon by reperfusion using leukocyte-depleted blood during direct percutaneous transluminal coronary angioplasty for acute mycardial infarction. J Cardiol 1999;33:145–152.

3 Jandl JH (ed): Granulocytes; in Blood: Textbook of Hematology, ed 2. Lippincott Williams & Wilkins, Philadelphia, 1996.

4 Krishnadasan B, Griscavage-Ennis J, Aldea GS, Verrier ED: Reperfusion injury during cardiopulmonary bypass; in Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology. Basel, Karger, 2002, pp 54–77.

5 Bevilaqua MP, Nelson RM: Selectins. J Clin Invest 1993;91:379–387.

6 Stewart M, Thiel M, Hogg N: Leukocyte integrins. Curr Opin Cell Biol 1995;7:690–696.

7 Springer TA: Traffic signals for lymphocyte recirculation and leukocyte emigration: The multistep paradigm. Cell 1994;76:301–314.

8 Weiss SJ: Tissue destruction by neutrophils. N Engl J Med 1989;320:365–376.

9 Asimakopoulos G: Mechanisms of the systemic inflammatory response syndrome. Perfusion 1999;14:269–277.

10 Van Eeden S, Miyagashima R, Haley L, Hogg JC: L-selectin expression increases on peripheral blood polymorphonuclear leukocytes during active marrow release. Am J Respir Crit Care Med 1995;151:500–507.

11 Boyle EM Jr, Pohlman TH, Johnson MC, Verrier ED: The systemic inflammatory response. Ann Thorac Surg 1997;64:S31–S37.

12 Frering B, Phillip I, Dehoux M, Rolland C, Langlois JM, Desmonts JM: Circulating cytokines in patients undergoing normothermic cardiopulmonary bypass. J Thorac Cardiovasc Surg 1994; 108:636–641.

13 John AE, Galea J, Francis SE, Holt CM, Finn A: Interleukin-8 mRNA expression in circulating leukocytes during cardiopulmonary bypass. Perfusion 1998;13:409–417.

14 Matata BM, Sosnowski AW, Galinanes M: Off-pump bypass graft operation significantly reduces oxidative stress and inflammation. Ann Thorac Surg 2000;69:785–791.

15 Ascione R, Lloyd CT, Underwood MJ, Lotto AA, Pitsis AA, Angelini GD: Inflammatory response after coronary revascularization with or without cardiopulmonary bypass. Ann Thorac Surg 2000; 69:1198–1204.

16 Bugajski P, Kalawski R, Zurawska I, Bartkowiak M, Wysocki H, Olszewski R, Siminiak T: Endothelin-1 is involved in plasma mediated stimulation of neutrophil adherence during coronary artery bypass grafting. Eur J Cardiothorac Surg 1999;15:408–412.

17 Ihnken K, Winkler A, Schlensak C, Sarai K, Neidhart G, Unkelbach U, Mulsch A, Sewell A: Normoxic cardiopulmonary bypass reduces oxidative myocardial damage and nitric oxide during cardiac operations in the adult. J Thorac Cardiovasc Surg 1998;116:327–334.

18 Kawamura T, Wakusawa R, Okada K, Inada S: Elevation of cytokines during open heart surgery with cardiopulmonary bypass: Participation of interleukin 8 and 6 in reperfusion injury. Can J Anaesth 1993;40:1016–1021.

19 Kawahito K, Kobayashi E, Ohmori M, Harada K, Kitoh Y, Fujimura A, Fuse K: Enhanced responsiveness of circulatory neutrophils after cardiopulmonary bypass: increased aggregability and superoxide producing capacity. Artif Organs 2000;24:37–42.

20 Patrick DA, Moore EE, Fullerton DA, Barnett CC, Meldrum DR, Silliman CC: Cardiopulmonary bypass renders patients at risk for multiple organ failure via early neutrophil priming and late neutrophil disability. J Surg Res 1999;86:42–49.

21 Asimakopoulos G, Taylor KM: The effects of cardiopulmonary bypass on leukocyte and endothelial adhesion molecules (current review). Ann Thorac Surg 1998;66:2135–2144.

22 Asimakopoulos G, Kohn A, Stefanou DC, Haskard DO, Landis RC, Taylor KM: Leukocyte integrin expression in patients undergoing cardiopulmonary bypass. Ann Thorac Surg 2000;69: 1192–1197.

23 Asimakopoulos G, Smith PL, Ratnatunga CP, Taylor KM: Pulmonary dysfunction and adult respiratory distress syndrome after cardiopulmonary bypass (current review). Ann Thorac Surg 1999;68:1107–1115.
24 Hashimoto K, Miyamoto H, Suzuki K, Horikoshi S, Matsui M, Arai T, Kurosawa H: Evidence of organ damage after cardiopulmonary bypass. The role of elastase and vasoactive mediators. J Thorac Cardiovasc Surg 1992;104:666–673.
25 Braude S, Nolop KB, Fleming JS, Krausz T, Taylor KM, Royston D: Increased pulmonary transvascular protein flux after canine cardiopulmonary bypass. Am Rev Respir Dis 1986;134:867–872.
26 Jorens PG, Van Damme J, De Backer W, Bossaert L, De Jongh RF, Herman AG, Rampart M: Interleukin-8 in the bronchoalveolar lavage fluid from patients with the adult respiratory distress syndrome (ARDS) and patients at risk for ARDS. Cytokine 1992;4:592–597.
27 Gu YJ, Wang YS, Chiang BY, Gao XD, Ye CX, Wildevuur CRH: Membrane oxygenator prevents lung reperfusion injury in canine cardiopulmonary bypass. Ann Thorac Surg 1991;51:573–578.
28 Turkoz R, Yorukoglu K, Akcay A, Yilik L, Baltalarli A, Karahan N, Adanir T, Sagban M: The effect of pentoxifylline on the lung during cardiopulmonary bypass. Eur J Cardiothorac Surg 1996;10: 339–346.
29 Hachida M, Hanayama N, Okamura T et al: The role of leukocyte depletion in reducing injury to myocardium and lung during cardiopulmonary bypass. ASAIO J 1995;41:M291–M294.
30 Johnson D, Thomson D, Hurst T, Prasad K, Wilson T, Murphy F, Saxena A, Mayers I: Neutrophil-mediated acute lung injury after extracorporeal perfusion. J Thorac Cardiovasc Surg 1994;107: 1193–1202.
31 Carney DE, Lutz CJ, Picone AL, Gatto LA, Ramamurthy NS, Golub LM, Simon SR, Searles B, Paskanin A, Snyder K, Finck C, Schiller HJ, Nieman GF: Matrix metalloproteinase inhibitor prevents acute lung injury after cardiopulmonary bypass. Circulation 1999;100:400–406.
32 Yamazaki T, Ooshima H, Usui A, Watanabe T, Yasuura K: Protective effects of ONO-5046*Ma, a specific neutrophil elastase inhibitor, on postoperative lung injury. Ann Thorac Surg 1999;68: 2141–2146.
33 Friedman M, Wang SY, Sellke FW, Franklin A, Weintraub RM, Johnson RG: Pulmonary injury after total or partial cardiopulmonary bypass with thromboxane synthesis inhibition. Ann Thorac Surg 1995;59:598–603.
34 Dreyer WJ, Michael LH, Millman EE, Berens KL, Geske RS: Neutrophil sequestration and pulmonary dysfunction in a canine model of open heart surgery with cardiopulmonary bypass. Evidence for a CD18-dependent mechanism. Circulation 1995;92:2276–2283.
35 Zahler S, Massoudy P, Hartl H, Hahnel C, Meisner H, Becker BF: Acute cardiac inflammatory responses to postischaemic reperfusion during cardiopulmonary bypass. Cardiovasc Res 1999;41: 722–730.
36 Wilson IC, Gardner TJ, DiNatale JM, Gillinov AM, Curtis WE, Cameron DE: Temporary leukocyte depletion reduces ventricular dysfunction during prolonged postischemic reperfusion. J Thorac Cardiovasc Surg 1993;106:805–810.
37 Hennein HA, Ebba H, Rodriguez JL, Merrick SH, Keith FM, Bronstein MH, Leung JM, Mangano DT, Greenfield LJ, Rankin JS: Relationship of the proinflammatory cytokines to myocardial ischemia and dysfunction after uncomplicated coronary revascularization. J Thorac Cardiovasc Surg 1994;108:626–635.
38 Neumann FJ, Ott I, Wilhelm A, Katus H, Tillmanns H, Schoming A: Release of chemoattractants and neutrophil activation in acute myocardial infarction immediately after successful recanalization of the infarct-related vessel by angioplasty. Eur Heart J 1999;15:171–178.
39 Azar RR, McKay RG, Kiernan FJ, Seecharran B, Feng YJ, Fram DB, Wu AH, Waters DD: Coronary angioplasty induces a systemic inflammatory response. Am J Cardiol 1997;80: 1476–1478.
40 Siminiak T, O'Gorman DJ, Shahi M, Hackett D, Sheridan DJ: Plasma-mediated neutrophil stimulation during coronary angioplasty: Autocrine effect of platelet activating factor. Br Heart J 1995;74:625–630.
41 Ikeda H, Nakayama H, Oda T, Kuwano K, Yamaga A, Ueno T, Yoh M, Hiyamuta K, Koga Y, Toshima H: Neutrophil activation after percutaneous transluminal coronary angioplasty. Am Heart J 1994;128(6 Pt 1):1091–1098.

42 Serrano CV Jr, Ramires JA, Venturinelli M, Arie S, D'Amico E, Zweier JL, Pileggi F, da Luz PL: Coronary angioplasty results in leukocyte and platelet activation with adhesion molecule expression. Evidence of inflammatory responses in coronary angioplasty. J Am Coll Cardiol 1997;29: 1276–1283.
43 Inoue T, Sakai Y, Fujito T, Hoshi K, Hayashi T, Takayanagi K, Morooka S: Clinical significance of neutrophil adhesion molecule expression after coronary angioplasty on the development of restenosis. Thromb Haemost 1998;79:54–58.
44 Kowalski J, Kosmider M, Pawlicki L, Glowacka E, Banasik M, Baj Z, Ciecwierz J, Pasnik J: Complement activates neutrophils during PTCA procedure in patients with unstable angina pectoris. Int J Cardiol 1997;58:229–240.
45 Mickelson JK, Lakkis NM, Villarreal-Levy G, Hughes BJ, Smith CW: Leukocyte activation with platelet adhesion after coronary angioplasty: a mechanism for recurrent disease? J Am Coll Cardiol 1996;28:345–353.
46 Roque M, Fallon JT, Badimon JJ, Zhang WX, Taubman MB, Reis ED: Mouse model of femoral artery denudation injury associated with the rapid accumulation of adhesion molecules on the luminal surface and recruitment of neutrophils. Arterioscler Thromb Vasc Biol 2000;20:335–342.
47 Easthham RD, Slade RR: Peripheral white blood cells; in Clinical Haematology, ed 7, London, Butterworth-Heinemann, 1992, pp 83–105.
48 Toft P, Nielsen CH, Tonnesen E, Hansen TG, Hokland M: Changes in adhesion molecule expression and oxidative burst activity of granulocytes and monocytes during open-heart surgery with cardiopulmonary bypass compared with abdominal surgery. Eur J Anaesthesiol 1998;15:345–353.
49 Kawahito K, Kawakami M, Fujiwara T, Adachi H, Ino T: Interleukin-8 and monocyte chemotactic activating factor responses to cardiopulmonary bypass. J Thorac Cardiovasc Surg 1995;110: 99–102.
50 Fingerle-Rowson G, Auers J, Kreuzer E, Labeta M, Schmidt B, Samtleben W, Ziegler-Heitbrock HW, Blumenstein M: Down-regulation of surface monocyte lipopolysaccharide-receptor CD14 in patients on cardiopulmonary bypass undergoing aorta-coronary bypass operation. J Thorac Cardiovasc Surg 1998;115:1172–1178.
51 Ernofsson M, Thelin S, Siegbahn A: Monocyte tissue factor expression, cell activation, and thrombin formation during cardiopulmonary bypass: A clinical study. J Thorac Cardiovasc Surg 1997;113:576–584.
52 Kappelmayer et al. (1993)
53 Rinder CS, Rinder HM, Johnson K, Smith M, Lee DL, Tracey J, Polack G, Higgins P, Yeh CG, Smith BR: Role of C3 cleavage in monocyte activation during extracorporeal circulation. Circulation 1999;100:553–558.
54 Tsuchida M, Watanabe H, Watanabe T, Hirahara H, Haga M, Ohzeki H, Hayashi J, Miyamura H, Hirono T, Abo T, Eguchi S: Effect of cardiopulmonary bypass on cytokine release and adhesion molecule expression in alveolar macrophages. Preliminary report in six cases. Am J Respir Crit Care Med 1997;156:932–938.

Dr. T. Gourlay, Department of Cardiac Surgery, NHLI, Imperial College Medical School,
2nd Floor B Block, Hammersmith Hospital, DuCane Road, London, W12 ONN (UK)
Tel. +44 208 383 3137, Fax +44 208 749 9418, E-Mail t.gourlay@ic.ac.uk

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 13–15

Leukocyte-Mediated Cerebral Disorders during and after Cardiac Surgery

Martin Scholz, Gerhard Wimmer-Greinecker, Georg Matheis

Department of Thoracic and Cardiovascular Surgery, J.W. Goethe University,
Frankfurt, Germany

Cardiac surgery with cardiopulmonary bypass (CPB) is associated with postoperative cerebral disorders. It seems that the blood-brain barrier is more sensitive to the pathogenicity of cardiac surgery than the endothelium in other organs. Three major causative pathomechanisms are discussed: (1) the aberrant activation of the immune system and clotting cascade elicited by the contact of patient blood with artificial surfaces of the extracorporeal circuit; (2) the cardiopulmonary reperfusion injury that occurs after release of the aortic cross clamp, and (3) micro- or macroembolic events caused by gas bubbles, biological aggregates, atheromatous debris, or inorganic matter, generated by CPB and/or mechanical stress including surgical manipulation (see Matheis and Scholz [1] in this book). These pathogenic factors may be associated with perioperative cerebral edema formation, and neurological sequelae of cardiac surgery. It has been shown that postoperative neuropsychological complications occur in 24–52% of cases, and that 0.4–5.2% of patients suffer from stroke. Moreover, MRI revealed that 100% of patients probably developed cerebral edema following cardiac surgery with CPB [2, 3].

Increased serum levels of S100B, a protein that is abundantly expressed in astroglia, and of the neuron-specific enolase (NSE) released by necrotic neuronal tissue, are found at the end of surgery [4, 5]. In most routine cases, S100B and NSE serum concentrations decline rapidly and reach baseline values within 1 or 2 days after surgery. It has been proposed that persistently elevated levels of S100B in serum are associated with a dysfunction of the blood-brain barrier and neurological disorders. In contrast, it has been reported that S100B serum levels significantly correlated with duration of CPB, but these patients did not necessarily exhibit neurological signs or symptoms, suggesting subclinical

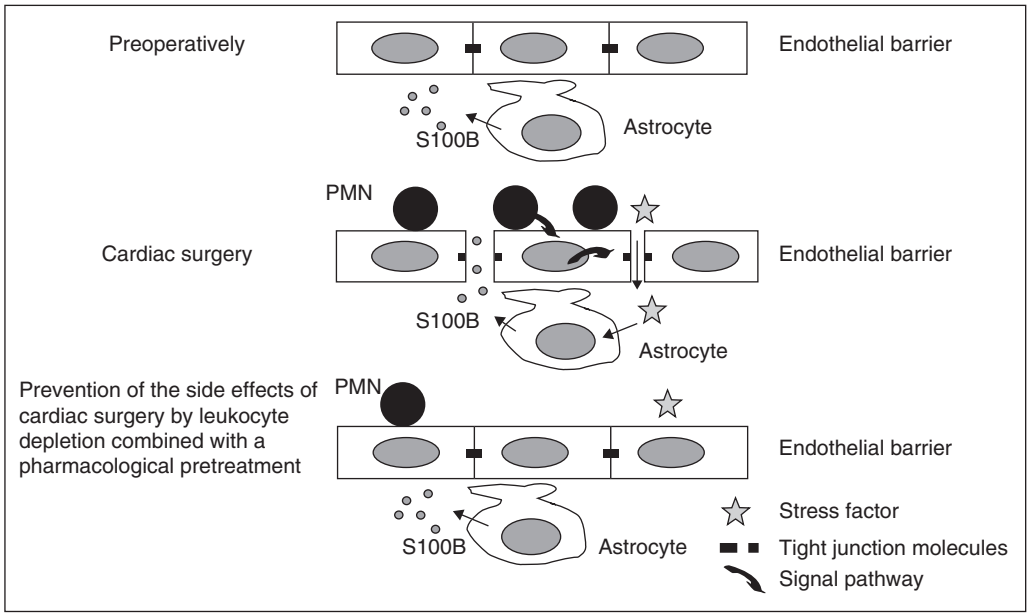


Fig. 1. Hypothesized pathomechanism on the level of the blood-brain barrier during/after cardiac surgery. Astrocytes are in the direct vicinity of the endothelial cells and ensure the integrity of the barrier. *Preoperatively*: tight junctions are functional and no S100B diffusion in the blood is observed. *Cardiac surgery*: activated polymorphonuclear leukocytes (PMN) adhere to the endothelium, secrete proinflammatory cytokines such as TNF-α, enzymes such as elastase or metalloproteinases and reactive oxygen specimens. For example, elastase may digest extracellular matrix proteins and intercellular adhesion molecules such as cadherins. In addition, signal transduction pathways may be activated that disturb the intracellular phosphorylation status of the tight junction molecules and thus the integrity of the barrier (arrows). Due to the leakage of the barrier, S100B can be measured in the blood stream. It is currently unclear how the above-mentioned stress factors manipulate the astrocytes, which are known to stabilize the barrier function. *Prevention of side effects of cardiac surgery*: the number of activated PMN has been reduced by leukocyte depletion. In addition, the tight junctions have been stabilized by pharmacological measures. No diffusion of S100B into the blood stream occurs.

cerebral injury or increased permeability of the blood-brain barrier [4]. It is likely that the etiology of cerebral injury and the rise in serum S100 levels are associated with cerebral microembolization. In addition, activated neutrophils that adhere to the vascular wall in the brain may disturb the integrity of the microvascular endothelium. However, the underlying pathomechanisms remain unknown and a concept to avoid cerebral swelling after CPB is needed. Figure 1 depicts a two-step model that might partly explain the leukocyte-mediated enhancement of S100B in serum. Activated leukocytes secrete proteinases that

may digest interendothelial zonula adherens molecules (cadherins) and molecules of the extracellular matrix. Moreover, leukocyte adhesion to endothelial cells involves intracellular signaling pathways that have been shown to modify the cytoskeleton and tight junction protein complexes by phosphorylation processes. These direct effects of neutrophils on the permeability of the blood-brain barrier might be an early event during CPB leading to unspecific diffusion of the astroglial product S100B into the blood stream. Astrocytes are known to ensure the integrity of the blood-brain barrier by yet undefined mechanisms and it can be proposed that leakage of the endothelium and thus the contact of these cells with reactive oxygen specimens, cytokines and neutrophils may also indirectly disturb the intercellular regulation of the blood-barrier function. Based on these findings we propose a combined treatment strategy consisting of leukocyte depletion (see the contribution of Matheis et al. [6] in this book) to reduce the pathogenic potential of neutrophils and a pharmacologically induced stabilization of the tight junctions (see the chapter by Berg et al. [7] in this book). This combined approach will hopefully improve outcomes in CPB surgery. Experiments are under way to confirm our expectations.

References

1 Matheis G, Scholz M: Pathogenicity of cardiopulmonary bypass and concepts of leukocyte filtration; in Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology. Basel, Karger, 2002, pp 47–53.

2 Harris DN, Bailey SM, Smith PL, Taylor KM, Oatridge A, Bydder GM: Brain swelling in first hour after coronary artery bypass surgery Lancet 1993;342:586–587.

3 Harris DN, Oatridge A, Dob D, Smith PL, Taylor KM, Bydder GM: Cerebral swelling after normothermic cardiopulmonary bypass. Anesthesiology 1998;88:340–345.

4 Westaby S, Johnsson P, Parry AJ, Blomqvist S, Solem JO, Alling C, Pillai R, Taggart DP, Grebenik C, Stahl E: Serum S100 protein: A potential marker for cerebral events during cardiopulmonary bypass. Ann Thorac Surg 1996;61:88–92.

5 Wimmer-Greinecker G, Matheis G, Brieden M, Dietrich M, Oremek G, Westphal K, Winkelmann BR, Moritz A: Neuropsychological changes after cardiopulmonary bypass for coronary artery bypass grafting. Thorac Cardiovasc Surg 1998;46:207–212.

6 Matheis G, Simon A, Scholz M: Strategic leukocyte filtration: Clinical and experimental experience; in Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology. Basel, Karger, 2002, pp 101–110.

7 Berg M, Scholz M, Matheis G: Further perspectives to limit the pathogenicity of leukocytes in cardiac surgery and cardiology; in Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology. Basel, Karger, 2002, pp 147–153.

PD Dr. Martin Scholz, Department of Thoracic and Cardiovascular Surgery,
J.W. Goethe University, Theodor-Stern-Kai 7, D–60590 Frankfurt (Germany)
Tel. +49 69 6301 6526, Fax +49 69 6301 7108, E-Mail m.scholz@em.uni-frankfurt.de

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 16–32

Mechanisms and Technical Aspects of Leukocyte Depletion

Stuart V. Sheppard

Southampton University Hospital, Southampton, UK

The concept of using filtration to remove leukocytes from blood is not new. Indeed as early as 1928 Alexander Fleming described a laboratory technique for the removal of leukocytes from small quantities of blood using cotton wool [1]. In 1962 the effect of using different types of fibres on cell removal was investigated [2]. This demonstrated that the use of Nylon fibres was at this time the most efficient method for removing granulocytes from heparinised blood. In 1972 the first commercially available filter was introduced for the depletion of leukocytes from red cell concentrates. This filter was constructed of a column tightly packed with cotton wool and sterilised.

This method of filtration, termed depth filtration does not restrict the removal of particles to the first blood contact surface as seen during screen filtration. Depth filters are constructed from rigid casings packed tightly with fibrous material [3], resulting in a structure with a wide distribution of pore sizes throughout the whole length of the filter. Particles are retained within the filter by either mechanical sieving or adhesion.

Mechanical removal of particles occurs by a number of mechanisms, particles can be both intercepted and trapped by smaller pores formed between a number of fibres, as seen during screen filtration, or particles become trapped in the area where two fibres cross or at the ends of individual fibres within the filter.

The adhesion of cells to the filter surface can also be influenced by a number of factors. Firstly the capacity of the filter media for cell adhesion, and secondly the effect of mechanical forces, such as gravity or blood flow encouraging cells to come into contact with the surface of the fibres to which they are then able to adhere. The surface charge of the fibre material also plays a role in attracting leukocytes towards the fibre surface.

The removal of leukocytes from blood by filtration may be either transient or permanent [4], and it is possible for trapped particles to become detached and pass through, and subsequently out of the filter.

The selection of different types of fibres, with respect to surface charges, chemistry and hydrophilicity can also influence the amount of leukocyte depletion occurring [5, 6]. The depletion of leukocytes from blood also depends on the physical properties of the blood as it is being filtered, due in part to cell-to-cell interactions [7–10], blood temperature [8, 11, 12], and the time elapsed since blood donation [13–15].

The choice of a fibre material for the depletion of leukocytes will depend on the physicochemical properties of the fibres: namely, surface charge, surface wettability, and surface chemistry. When developing new fibres it is not easy to directly assess the effect that changing one individual property will have on its leukocyte depletion efficacy. Primarily because a change in surface chemistry may also lead to changes in the wettability of the fibres and even the surface charge present at the blood-fibre interface, either of which may further alter the leukocyte-depleting properties of the fibres.

The effect that surface charge has on the deposition of cells to the fibre surface will depend on an energy barrier induced by electrostatic interactions and London-van der Walls forces. Normally leukocytes have a negative surface charge, and it has been postulated that the efficiency of a leukocyte-depleting filter is reduced if the surface charge of the fibres is more negative. Interestingly, it has been demonstrated [16] that coating the surface of the fibres with diethyl-amino-ethyl-methacrylate (DEAEMA) increases the positive charge on the fibres sufficiently to cause preferential binding of leukocytes. The optimal concentration of DEAEMA for leukocyte removal was found to be 5%; however, increasing the concentration above this led to adhesion of both leukocytes and platelets to the fibre surface.

The hydrophilicity or wettability of the fibre surface results from the interplay between the forces occurring between the fibre surface and the liquid; this is referred to as the interfacial surface tension. Synthetic fibres have a surface tension lower than that of blood and when the surface tension of the fibre is substantially lower than that of the fluid, beading of fluid occurs and thus, the fibre surface cannot be ‘wetted’ adequately. Adequate wetting of the fibres is important in ensuring optimal contact and subsequent adhesion of the leukocytes to the fibre surface and thus, optimal filtration will only be possible if the entire surface of the fibres is exposed to the blood. Enhancing the hydrophilicity of the fibre material to ensure efficient priming of the device will ensure that all air is completely evacuated. Residual pockets of air will lead to uneven distribution of blood flow through the device during filtration and hence reduce the efficiency of cell removal. Although hydrophobic surfaces allow leukocyte

adhesion [17, 18], it has been postulated that hydrophilic surfaces demonstrate better adhesion for leukocytes [6, 19].

Current Leukocyte Depletion Technologies

The design of characteristics of modern leukocyte-depleting filters is rapidly becoming a science in its own right. With the development of leukocyte-reducing filters for specific applications such as the re-infusion of salvaged blood or leukocyte-depleted blood cardioplegia. Even the amount of leukocyte depletion required varies from one application to another. These variations are achieved by changing either the physical or biological forces affecting leukocyte retention within each type of filter as previously discussed.

Typically, modern leukocyte-depleting filters are constructed from a rigid housing containing a synthetic medium specifically designed for the retention of leukocytes. The blood flow characteristics of the filter are designed to ensure that blood passing through the device receives maximal contact with the filter media. This is achieved during the design stage using computers to evaluate fully and perfect the blood flow profile through each device before full production.

Another consideration addressed during this design phase is the ability to effectively expel all the air from the device and allow efficient priming.

Mechanisms of Leukocyte Reduction by Filters

Broadly speaking, leukocyte-depleting filters can be divided into two types. The first relies on porous foam for leukocyte removal while the second type uses fibre technology for the removal of leukocytes.

The efficacy of porous foam for the removal of leukocytes has been shown [20] to demonstrate a linear relationship between the thickness of the porous material and the reduction rate for leukocytes. The authors also found that the deformability of different blood cells may play an important role in their entrapment and subsequent evacuation from the capture sites within the foam. This suggests that the difference in deformability between leukocytes and erythrocytes may be a major reason for the selective removal of leukocytes seen with porous materials. Interestingly this type of filter has been shown to be more efficient as the temperature of the blood is reduced, which further supports the argument that cellular deformability plays a major role in the leukocyte depletion associated with this type of filter. When sodium azide was added

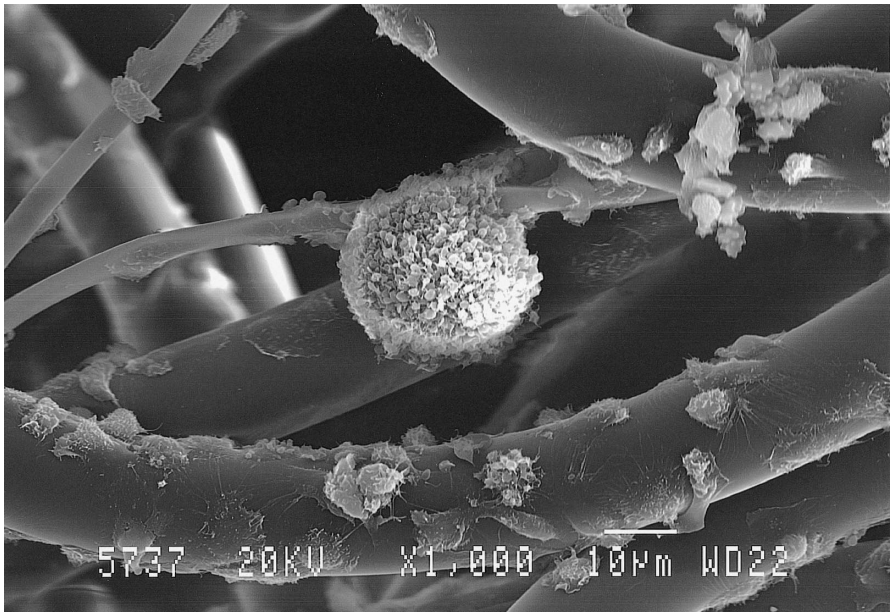


Fig. 1. This scanning electron micrograph taken at ×1000 magnification shows a leukocyte adhered to the fibre surface at the 'cross-over' point between two fibres.

to diminish leukocyte metabolism, the rate of leukocyte removal increased, suggesting that leukocytes behave as non-biological particles in this type of filter.

The mechanism of leukocyte removal with fibres has also been shown to have a linear relationship with the thickness of the non-woven fabric. Altering the average fibre diameter contained within the filter while still maintaining the same packing density of the fibre compartment can vary the extent of leukocyte removal. It has been demonstrated [21] that when the average fibre diameter was reduced from 1.7 to 1.2 μm the amount of leukocyte depletion doubled. Since both fabrics used the same packing density, the length of the fibres contained within one unit volume of fabric of 1.2 μm is calculated to be twice that of 1.7 μm. This size reduction will give a large increase in the number of points where two or more fibres are in contact with each other 'cross-over points' which constitute a substantial site for leukocyte adsorption (fig. 1). When leukocyte metabolism was diminished by the addition of sodium azide the removal rate was also reduced, suggesting that there is an element of active cell adhesion when using fibres for leukocyte depletion.

Leukocyte Depletion during Cardiopulmonary Bypass

The first purpose-built filter for the removal of leukocytes from the arterial line of the extracorporeal circuit was the Leukoguard LG6 (Pall Biomedical, New York, N.Y., USA), which was introduced in 1992. This was a modified type of arterial line filter that combined a 40 μm polyester screen filter with a surface-modified fibre bundle for the specific removal of leukocytes. Early laboratory studies [22] using bovine blood demonstrated that the filter had good air handling characteristics similar to the non-leukocyte-depleting filters available at that time. Platelet depletion, trans-filter pressure gradient and cellular trauma caused by the filter were found to be similar to other models available. Efficacy of leukocyte removal was assessed using manual counting techniques, demonstrating that over the course of a 90-min experiment the filter removed around 50% of the total leukocytes and achieved 70% depletion of neutrophils. The same group was also able to demonstrate [23] that the filter performed equally well when blood was delivered using either pulsatile or continuous flow. Significant depletion of neutrophils has also been shown to occur in human blood [24]. Using leukocyte-associated monoclonal antibodies to measure the expression of neutrophil activation antigens, the same group postulated [25] that the leukocyte-depleting filter selectively removed activated neutrophils, but in the clinical setting there was no significant reduction in the total leukocyte count. This supported their concept that the filter preferentially removed activated neutrophils.

The trans-filter pressure gradient has been assessed in the clinical setting and found to be slightly higher than that of a standard non-leukocyte-depleting filter [26], as one would expect due to the presence of an additional fibre pack. Although the pressure gradient measured was higher, it was not felt to be clinically significant at any of the flow rates investigated. It was interesting, however, that the pressure drop was seen to increase with the duration of cardiopulmonary bypass (CPB) in both types of filter. However, this increase in pressure with time is significantly greater from 30 min onwards (fig. 2).

It has been demonstrated [27] that both total and activated leukocyte counts increase significantly following removal of the aortic cross-clamp. The steeper increase in the pressure drop across the leukocyte-depleting filter after the 30-min time point may be due to the accumulation of leukocytes on the fibres within the filter thus affecting the blood flow through the device.

There has been conflicting evidence as to the benefits of leukocyte depletion in man. The most convincing evidence came from early laboratory studies using animals, which may not truly reflect clinical CPB in man. In some of the published clinical studies, however, too few patients have been evaluated and so the ability to detect the difference in main measured variables had a power [28] as low as 0.3.

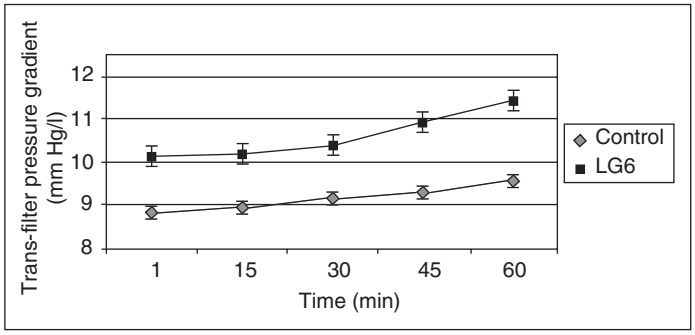


Fig. 2. Changes in pressure drop across the LG6 filter during CPB.

Furthermore, in the absence of a leukocyte-depleting filter, arterial oxygen tension (PaO_2) has been shown to return to normal within 7 h after CPB [29]. In one study [30] the pulmonary index was worse in the control group in patients not receiving a leukocyte-depleting filter for up to 24 h after CPB. However, another study [31] found no clinical difference in respiratory function between either group, while another [32] found the early difference had disappeared by 24 h. Myocardial function in leukocyte-depleted patients has been demonstrated to be better [30, 33] with lower creatine kinase-MB (CK-MB) concentrations and less inotrope requirements, when compared to non-leukocyte-depleted controls. A number of workers [31, 34], however, have failed to demonstrate any improvement in postoperative lung function, pulmonary vascular resistance or ventilation time when using leukocyte depleting filters.

Interestingly the duration of CPB has also been shown [35] to influence the efficacy of leukocyte depletion, no improvement in pulmonary status was demonstrated when the duration of CPB was less than 90 min. On reviewing the published literature it is apparent that previous studies which demonstrated a positive benefit using leukocyte depletion all had a CPB duration in excess of 90 min while those who were unable to show a benefit had significantly shorter bypass times (table 1).

Although the duration of CPB may influence the ability to detect gross changes in pulmonary function and other clinical markers of efficacy, the blood temperature has also been shown to affect the level of leukocyte depletion occurring both in vitro [39] and in vivo [40]. In the laboratory setting the LG6 filter media was shown to remove leukocytes across a range of blood temperatures (fig. 3). However, there was a significant reduction in the amount of leukocytes that were depleted as the blood temperature was decreased.

In none of these studies, however, did the decreased blood temperature affect the ability of the filter to rapidly remove activated leukocytes from the

Table 1. Summary of some of the previously reported clinical results relative to duration of CPB in the study

Reference	Year	Study design	Benefit Y/N	CPB time
Palanzo et al. [36]	1993	elective cases 18 leukocyte-depleted 18 control	Y	> 90 min
Hachida et al. [30]	1995	elective cases 14 leukocyte-depleted 14 control cases	Y	> 180 min
Mihalijevic et al. [31]	1995	14 leukocyte-depleted 18 control cases	N	< 90 min
Thurlow et al. [25]	1996	7 leukocyte-depleted 7 control cases	N	< 90 min and a small sample size
Kennedy et al. [37]	1997	elective cases 100 leukocyte-depleted 100 control cases	N	< 90 min
Sheppard et al. [26]	1997	elective cases 50 leukocyte-depleted 50 control cases	Y	> 90 min
Baksaas et al. [38]	1998	20 leukocyte-depleted 20 control cases	N	< 90 min

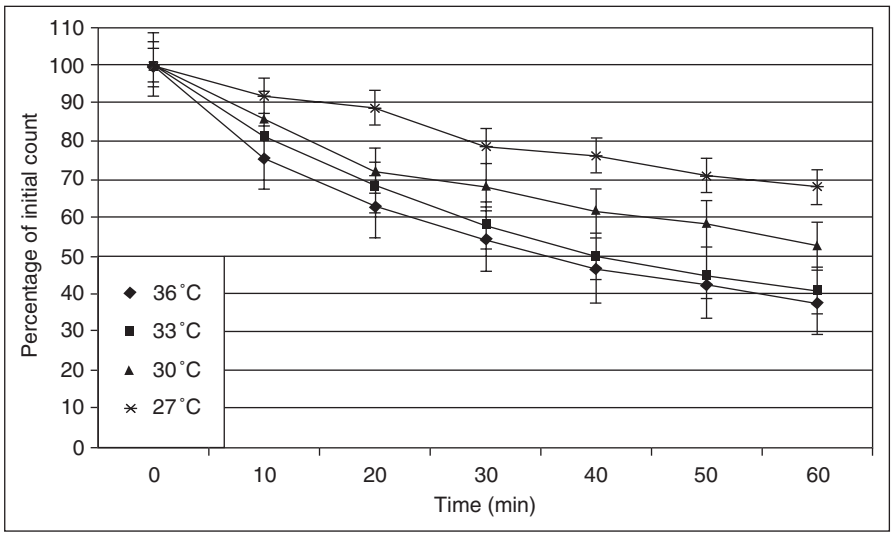


Fig. 3. Changes in total leukocyte count in the in vitro circuit expressed as the change from baseline values.

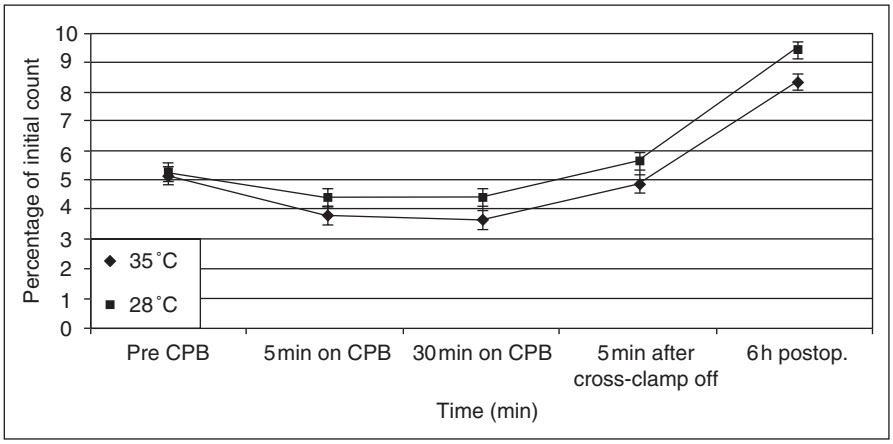


Fig. 4. Changes in total leukocyte count during CPB using blood temperatures of 28 °C and 35 °C.

circulating blood either during the initial stages of filtration or after the filter had been running for some time.

In the clinical setting a similar phenomenon has been demonstrated [40], with more leukocyte depletion occurring when CPB was conducted at 35 °C and compared to CPB at 28 °C, as shown in figure 4.

Alternative Methods of Systemic Leukocyte Depletion

More recently a number of different methods for applying leukocyte depletion have been described. Firstly, a method of leukocyte depletion using a venous line bypass loop has been described [41]. For this technique an additional loop of tubing between the venous line and the cardiotomy reservoir, containing two leukocyte-depleting filters (J1647, Pall Corporation) was inserted into the extracorporeal circuit. A section of the loop was placed in a roller pump and blood was then circulated through the filters at a flow rate of 400 ml/min for 10 min, thus filtering a volume of 2,000 ml per filter and a total volume of 4,000 ml of perfusate. Filtration was commenced after the start of systemic rewarming but prior to removal of the aortic cross-clamp. Using this technique a 38% reduction in the total number of circulating neutrophils was achieved at the point of aortic cross-clamp removal.

An alternative technique for employing leukocyte depletion in the arterial limb of the circuit has been described [42]. During this technique the leukocyte-depleting filter is sited in the arterial line in parallel with a standard screen

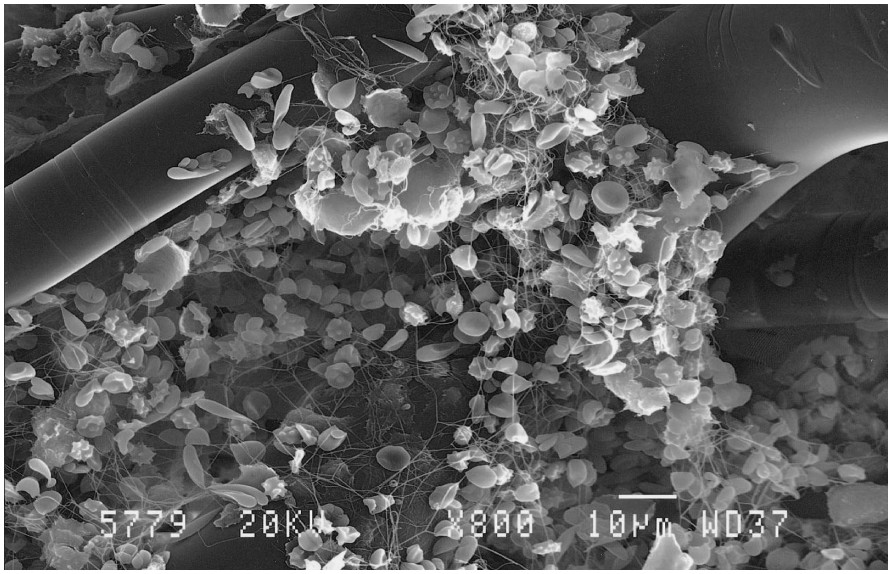


Fig. 5. Scanning electron micrograph taken of a blocked leukocyte-depleting filter after rapid blood cooling. Note how clean the fibres are and the tight fibrin network with large amounts of trapped cells.

filter. Leukocyte depletion is not employed until the later stages of CPB deploying the filter just before the removal of the aortic cross clamp and continuing for the first 15 min of reperfusion; so-called 'strategic leukodepletion'. Clinically this demonstrated lower levels of troponin T in the leukocyte-depleted group, indicating a reduction in myocardial damage.

Alternate strategies may be developed in response to the small incidence of filter occlusion that has been seen associated with leukocyte-depleting filters. This phenomenon may be similar to that seen during the use of membrane oxygenators in CPB. Although the cause of this problem has yet to be fully described, the current literature [43] suggests that there are a small number of patients who have some form of a pro-coagulant activity during CPB. This may lead to the formation of fibrin and/or deposition of platelets on the surfaces of the extracorporeal circuit which in turn reduces the size of the blood path through the filter and in some cases this is sufficient to cause an irreversible increase in the pressure gradient. Research would suggest that this problem can be exacerbated by rapid cooling of blood to temperatures below 20 °C [44]. An in vitro study was able to demonstrate filter occlusion under these circumstances was due to the accumulation of a fibrin network throughout the fibres of the filter, leading to entrapment of cells and subsequent filter occlusion as seen in figure 5.

Leukocyte Depletion of Blood Cardioplegia

Blood cardioplegia is now widely used for myocardial protection during periods of surgical ischaemia. Whilst blood cardioplegia is generally considered to provide superior myocardial protection to crystalloid with regard to oxygen delivery and to possess more physiological properties, the presence of leukocytes and platelets can cause reperfusion injury and capillary plugging.

A number of animal experiments have demonstrated that leukocyte depletion of blood cardioplegia reduces the amount of myocardial injury and can improve cardiac function. In one such study [45] using isolated canine hearts a significant increase in recruitable stroke work and cardiac output in the leukocyte-depleted group was seen even after 24 h hypothermic preservation. Further studies [46, 47] using isolated lamb and neonatal pig hearts were also able to demonstrate better recovery of both mechanical and endothelial function in the leukocyte-depleted group. When leukocytes were not depleted the stroke work index was significantly reduced and necrosis, mitochondrial disruption, nuclear clumping and interstitial oedema were present.

Most of these animal studies used leukocyte depletion transfusion filters to remove the leukocytes from the blood used for cardioplegia. Blood was taken from the CPB circuit and then passed through the leukocyte filter into a collection reservoir that was then used to supply blood cardioplegia as required. Whilst this technique was successful in these animal models, the use of transfusion filters in the clinical situation is less than ideal, particularly as transfusion filters are not designed to work with blood pumps and have a limited filtering capacity. The Pall RC100 (Pall Corporation) was designed to filter one, possibly two, units of blood containing 350 ml each using a flow rate of less than 100 ml/min.

It should also be noted that in addition to depleting leukocytes these transfusion filters were also capable of significantly reducing the platelet count in the blood being filtered. The filters were designed to handle stored red cell concentrates that contained non-viable platelets not required as part of the transfusion. This ability of this type of filter to remove platelets from the blood may also have been responsible for some of the benefits seen with its use.

During the early 1990s when this technique was in its infancy, there were no specific filters available for blood cardioplegia, and a number of workers began reporting clinical studies using transfusion filters, the results of which are summarised in table 2. Indeed at this time some centres still continue to use this as their preferred method of leukocyte reduction during cardioplegia administration.

It would appear that in the heart transplant setting, leukocyte reduction is associated with a reduction in ultrastructural evidence of reperfusion injury and associated release of CPK-MB and thromboxane B_2 [48]. Other studies have shown that markers of myocardial damage such as tropinin I, CPK-MB, lipid

Table 2. Summary of results obtained using transfusion filters for leukocyte depletion of cardioplegia

Reference	Year	Study design	Benefit Y/N	Filter type
Pearl et al. [48]	1992	transplant patients 16 control patients 16 leukocyte-depleted	Y	Pall RC100
Ichihara et al. [49]	1994	elective cases 12 control cases 12 leukocyte-depleted	Y	Pall RC100
Pala et al. [50]	1994	ejection fraction <35% 11 control cases 11 leukocyte-depleted	Y	unknown
Palatianos and Balentine [51]	1994	elective cases 30 control cases 49 leukocyte-depleted	Y	Pall RC400
Sawa et al. [52]	1996	ventricular hypertrophy 10 control cases 10 leukocyte-depleted	Y	Asahi Cellsorba 80P
De Vecchi et al. [53]	1997	ejection fraction >45% 11 control cases 11 leukocyte-depleted	N	Leukoseize 2
Palatianos et al. [54]	2000	160 consecutive cases 80 control cases 80 leukocyte-depleted	Y	Pall RC400

peroxides, and elastase are also reduced if leukocyte depletion of the blood cardioplegia is used [49, 51, 52] with most studies electing to provide leukocyte depletion during the reperfusion period (terminal cardioplegia). However, the most significant demonstration of improved myocardial protection and resulting improvement in cardiac performance was recently reported [54] using leukocyte filters for cardioplegia during the period of aortic cross clamping.

As an alternative to using leukocyte-depleting transfusion filters for blood cardioplegia there are now filters available which fit directly into the blood cardioplegia line and are designed to filter larger volumes of blood and run at higher blood flow rates in the order of 500 ml/min.

Sawa et al. [52] have reported the use of an extracorporeal leukocyte-depleting filter, the Asahi Cellsorba 80P (Asahi Medical, Tokyo, Japan). This contains a fabric of non-woven polyester fibres for the removal of leukocytes, has a priming volume of 80 ml and is capable of filtering heparinised blood at 300 ml/min for 10 min.

In their study, controlled myocardial reperfusion was achieved using a coupled double-head roller pump system with one roller pump supplying the crystalloid solution while the other pumped oxygenated blood through the Cellsorba 80P filter. The crystalloid solution was mixed with the leukocyte-depleted blood via a 'Y' connector sited just after the outlet of the filter. Terminal blood cardioplegia was administered via the aortic root for the first 10 min of myocardial reperfusion.

More recently the Leukoguard BC1 (Pall Corporation) has been introduced for the specific task of leukocyte-depleting blood cardioplegia. The device consists of a rigid case constructed from acrylic resin containing a 40-μm screen filter combined with a surface-modified non-woven polyester fabric for the specific removal of leukocytes from heparinised blood. It has a total priming volume of 220 ml and can be run with blood flow rates up to 500 ml/min.

A number of studies have now reported on the use of this filter for blood cardioplegia administration. A summary of study results is presented in table 3. Interestingly, while using the BC1B (Pall Corporation) for multiple dose cardioplegia administration, it has been reported [55] that the efficiency of leukocyte reduction significantly decreased after 1.5 litres of cardioplegia had been delivered. However, in a later paper the clinical efficacy of the filter demonstrated a reduction in tropinin I release, although the authors were using two BC1 filters during this study. This raises the question as to the extent of leukocyte removal required from a blood cardioplegia filter.

In the animal models discussed previously, filters giving >99% total leukocyte removal were used, whereas the efficiency of the BC1B (Pall Corporation) would seem to be initially >95% the depletion rate has been seen to decrease after between 1.5 and 3 litres of cardioplegia have been administered [55].

Unfortunately, to date there are no data on the efficacy of the BC1 filter to remove activated neutrophils. However, one could hypothesise that based on observations with the Leukoguard LG6 arterial line filter (Pall Corporation) discussed earlier in this chapter, the BC1 filter is probably capable of removing all activated neutrophils. It is not clear from current published clinical evidence whether an increase in the leukocyte removal efficiency would provide any further clinical improvements.

Positioning of Leukocyte-Depleting Cardioplegia Filters

It is recommended that the filter should be placed after the cardioplegia pump, but before the cardioplegia heat exchanger. The positioning of the filter before the heat exchanger is important as problems with increased trans-filter pressures have been reported when the filter was placed after the heat exchanger. Presumably, like the LG6 arterial line filter, high trans-filter pressure gradients

Table 3. Summary of previously published results using leukodepleted blood cardioplegia

Reference	Year	Study design	Benefit Y/N	Filter type
Sawa et al. [52]	1994	elective cases 15 whole blood 10 terminal cardioplegia 15 terminal cardioplegia with leukodepletion	N	Asahi Cellsorba 80P
Sawa et al. [52]	1994	emergency cases 10 whole blood 10 terminal cardioplegia 10 terminal cardioplegia with leukodepletion	Y	Asahi Cellsorba 80P
Roth et al. [55]	1997	16 leukocyte-depleted patients 8 with a single filter 8 with two filters	N	Pall Corporation BC1B
Heggie et al. [56]	1998	14 elective cases	Y	Pall Corporation BC1B
Suzuki et al. [57]	1998	elective cases 20 control 20 leukocyte-depleted	Y	Pall Corporation BC1B
Nakamea et al. [58]	1998	elective cases 5 control 5 leukocyte-depleted	Y	Pall Corporation BC1B
Roth et al. [59]	1999	32 cases 15 controls	Y	Pall Corporation 2 × BC1B
Browning et al. [60]	1999	40 cases 20 control 20 leukocyte-depleted	N	Pall Corporation BC1B
Matsuda et al. [61]	1999		Y	Pall Corporation BC1B
Hayashi et al. [62]	2000	paediatrics 25 control 25 leukodepleted	Y	Pall Corporation BC1B

may be experienced if the blood is cooled rapidly and precipitation of fibrin occurs.

A number of authors have reported siting the filter prior to the cardioplegia pump. Recent work [63] has demonstrated that leukocyte removal efficiency is not significantly affected if the filter is placed immediately before the cardioplegia pump.

Use of the BC1 filter in a re-circulation system has also been described [56]. The technique was found to remove more than 70% of leukocytes from

an average volume of 5.3 litres of blood cardioplegia, whilst only removing approximately 10% of platelets. Red cell haemolysis was negligible and a pressure drop of 10.8 mm Hg at a mean flow rate of 315 ml/min was reported using this technique.

The BC1 filter has been used during paediatric cardiac surgery to leukocyte deplete the cardioplegia administered during the ischaemic period, with leukocyte depletion rates of 90% reported using this technique [56].

It has been commented that that size of the BC1 filter (220 ml) adds considerably to the priming volume of the extracorporeal circuit especially for paediatric patients. Whilst a smaller filter may be more acceptable for current circuits, reducing the size of the filter will lead to a reduction in filter media available for blood contact. This may then lead to a decreased capacity for leukocyte removal and potentially increase the trans-filter pressure drop. Whilst the Asahi Cellsorba 80P has a smaller priming volume at 80 ml, the only clinical reports have come from Japan and there are no reports of its leukocyte-depleting efficiency during cardioplegia other than by Sawa et al. [52] who reported an 85–95% leukocyte reduction in the aortic root. It should be remembered, however, that no leukocyte counts were taken from the outlet of the filter and therefore the filter may be more efficient at removing leukocytes than first published.

Conclusion

The technique of leukocyte depletion has developed rapidly over the past decade with modern leukocyte-depleting filters now available for a whole range of dedicated tasks. Improvements in both filter design and leukocyte-removing filter media will allow this technique to be refined over the coming years. The majority of leukocyte-depleting filters currently marketed are based on fibre technology for the active removal of leukocytes. Improvements in fibre surface modification will enable us to achieve higher levels of cell removal using smaller devices, allowing the technique to become more accessible to neonatal and paediatric patients where priming volumes are a critical issue.

Research has shown that the use of leukocyte depletion can reduce reperfusion injury and hence provide improved cardiopulmonary status following CPB. It is not clear at present what level of leukocyte depletion is required to maximise these clinical benefits, or whether merely the removal of activated neutrophils would demonstrate equivalent benefits. Improvements in patient status have been reported using leukocyte depletion during the whole period of CPB, but there is a significant growth in evidence demonstrating similar clinical benefits following the strategic use of the technique confined to the reperfusion period.

Over the next decade our understanding of the exact mechanisms by which leukocytes are removed and the exact amount of depletion required to optimise clinical benefit will increase allowing further refinement of the technique.

References

1 Fleming A: A simple method of removing leukocytes from blood. Br J Exp Pathol 1928;7:281.
2 Greenwalt TJ, Gajewski M, McKenna JL: A new method for preparing buffy-coat poor blood. Transfusion 1962;2:221.
3 Swank RL, Hirsh H, Breur M, Hissen W: Effect of glass wool filtration on blood during extracorporeal circulation. Surg Gynecol Obstet 1963;117:547–552.
4 Forrester JV, Lackie JM: Adhesion of neutrophil leukocytes under conditions of flow. J Cell Sci 1984;70:93–110.
5 Absolom DR, Thompson C, Hawthorn, Zingg W, Neumann AW: Kinetics of cell adhesion to polymer surfaces. J Biomed Mater Res 1988;22:215–229.
6 Van Oss CJ, Gillman CF, Neumann AW: in Phagocytic Engulfment and Cell Adhesiveness as Cellular Surface Phenomena. New York, Dekker, 1975.
7 Loos JA, Blok-Schut B, Kipp B: Size distribution, electronic recognition and counting of human blood monocytes. Blood 1976;48:473.
8 Williamson JR, Shanahan MO, Hodimuth RM: The influence of temperature on red cell deformability. Blood 1975;46:611–624.
9 Milner GR, Fagence R, Darnborough J: Temperature dependence of leukocyte depletion of blood with an automatic blood cell processor. Transfusion 1982;22:48–50.
10 Albelda SM, Buck CA: Integrins and other cell adhesion molecules. FASEB J 1990;4:2868–2880.
11 Lederman DM, Cumming RD, Petschek HE, Levine PH, Krinsley NI: The effect of temperature on the interaction of platelets and leukocytes with materials exposed to flowing blood. Trans Am Soc Artif Intern Organs 1978;24:557–560.
12 Manara FS, Schneider DL: The activation of the human neutrophil respiratory burst occurs only at temperatures above 17°C: Evidence that activation requires membrane fusion. Biochem Biophys Res Commun 1985;132:696–701.
13 Swank RL: Alteration of blood on storage: Measurement of adhesiveness of ageing platelets and leukocytes and their removal by filtration. N Engl J Med 1961;265:728.
14 Truter EJ, Murray PW: Morphological classification by scanning electron microscopy of erythrocytes stored at 4°C in citrate-phosphate-dextrose anticoagulant. Med Lab Sci 1990;47:113–119.
15 Humbert JR, Fermin CD, Winsor EL: Early damage to granulocytes during storage. Semin Hematol 1991;28:10–13.
16 Dzik S: Mechanisms of leukocyte removal by filtration; in Lane TA, Myllylä G (eds): Leukocyte-Depleted Blood Products. Curr Stud Hematol Blood Transf. Basel, Karger, 1994, vol 60, pp 123–133.
17 Van Kampen CL, Gibbons DF: Effect of implant surface chemistry upon arterial thrombosis. J Biomed Mater Res 1979;13:517–541.
18 Lim F, Cooper SL: The effect of surface hydrophilicity on biomaterial-leukocyte interactions. ASAIO Trans 1991;37:M146–M147.
19 Unarska M, Robinson GB: Adherence of human leukocytes to synthetic polymer surfaces. Life Support Syst 1978;5:283.
20 Kora S, Kuroki H, Kido T, Katsurafda N, Sado M: Mechanisms of leukocyte removal by porous foam; in Clinical Applications of Leukocyte Depletion. Oxford, Blackwell Scientific, 1993.
21 Oka S, Maeda K, Nishimura T, Yamawaki N: Mechanisms of leukocyte removal by fibers; in Clinical Applications of Leukocyte Depletion. Oxford, Blackwell Scientific, 1993.
22 Gourlay T, Fleming J, Taylor KM: Laboratory evaluation of the Pall LG-6 leucocyte depleting arterial line filter. Perfusion 1992;7:131–140.

23 Gourlay T, Fleming J, Taylor KM: The effects of pulsatile flow on the leucocyte depleting qualities of the Pall LG-6 leucocyte depleting arterial line filter. Perfusion 1992;7:227–232.
24 Thurlow PJ, Doolan L, Sharp R, Sullivan M, Smith B: Studies of the effect of Pall leucocyte filters LG6 and AV6 in an in vitro simulated extracorporeal circulatory system. Perfusion 1995;10: 291–300.
25 Thurlow PJ, Doolan L, Sharp R, Sullivan M, Smith B, Andersen LW: Laboratory studies of the effect of Pall extracorporeal leucocyte filters LG6 and AV6 on patients undergoing coronary bypass grafts. Perfusion 1996;11:29–37.
26 Sheppard SV, Kennedy NJ, Smith DC: Arterial line filter pressure gradients. United Kingdom Congress on Perfusion, Nottingham, 1997.
27 JK Nanson, SV Sheppard, M Guryeta, DC Smith: A comparison of sequential total and activated white cell count in patients undergoing coronary artery bypass grafting, using cardiopulmonary bypass, with and without a white cell filter. Association of Cardiothoracic Anaesthetists, Glasgow, 1999.
28 Altman DG: How large a sample? in Gore SM, Altman DG (eds): Statistics in Practice. London, British Medical Journal, 1982.
29 Reeve WG, Ingram SM, Smith DC: Respiratory function after cardiopulmonary bypass: A comparison of bubble and membrane oxygenators. J Cardiothorac Vasc Anesth 1994;8:502–508.
30 Hachida M, Hanayama N, Okamura T, Akasawa T, Maeda T, Bonkohara Y, Endo M, Hashimoto A, Koyanagi H: The role of leukocyte depletion in reducing injury to myocardium and lung during cardiopulmonary bypass. ASAIO J 1995;41:M291–M294.
31 Mihaljevic T, Tonz M, Von-Segesser LK, Pasle M, Grob P, Fehr J, Seifert B, Turina M: The influence of leukocyte filtration during cardiopulmonary bypass on postoperative lung function: A clinical study. J Thorac Cardiovasc Surg 1995;109:1138–1145.
32 Johnson D, Thomson D, Mycyk T, Burbridge B, Mayers I: Depletion of neutrophils by filtration during aortocoronary bypass surgery transiently improves postoperative cardiorespiratory status. Chest 1995;107:1253–1259.
33 Chiba Y, Muraoka R, Haya AI, Morioka K, Sasaki M, Uesaka T: Leucocyte depletion and prevention of reperfusion injury during cardiopulmonary bypass: A clinical study. Cardiovasc Surg 1993; 1:350–356.
34 Al-Ebrahim K, Shaffei H: Our experience with using the Pall LG6 leukocyte depleting filter during cardiopulmonary bypass. Ann Thorac Surg 1993;56:1216–1217.
35 Sheppard SV, Rehman RH, Kennedy NJ, Smith DC: Does the duration of cardiopulmonary bypass influence the clinical effectiveness of leucocyte depleting arterial line filters. Association of Cardiothoracic Anaesthetists, London, 1998.
36 Palanzo DA, Manley NJ, Montesano RM, Yiesley GL, Gordon D: Clinical evaluation of the LeukoGuard (LG-6) arterial line filter for routine open heart surgery. Perfusion 1993;8:489–496.
37 Kennedy NJ, Sheppard SV, Smith DC: Leucocyte depleting vs non-leucocyte depleting filters. A prospective randomised study comparing oxygenation indices and time to extubation. Br J Anaesth 1998;80(2):43.
38 Baksaas ST, Videm V, Mollnes TE: Leucocyte filtration during cardiopulmonary bypass hardly changed leucocyte counts and did not influence myeloperoxidase, complement, cytokines or platelets. Perfusion 1998;13:429–436.
39 Sheppard SV, Smith DC, Gibbs RV: The effect of blood temperature on leucocyte depletion rates an in vitro study. 25th Anniversary Congress on Perfusion, Jersey, 1999.
40 Alexiou C, Sheppard SV, Haw MP, Patel JS, Smith DC, Gibbs RV, Barlow CW, Tsang GMK, Ohri SK, Livesey SA, Monro JL: The effect of blood temperature (35°C vs. 28°C) on leucocyte depletion during CABG. Therapeutic Applications of Leukocyte Filtration Meeting, London, 2000.
41 Gu YJ, de Vries AJ, Vos P, Boonstra PW, Van Oeveren W: Leukocyte depletion during cardiac operations: A new approach through the venous bypass circuit. Ann Thorac Surg 1999;67: 604–609.
42 Matheis G, Scholz M, Gerber J, Abdel-Rahman U, Wimmer-Greinecker G, Moritz A: Leukocyte filtration in the early reperfusion phase on cardiopulmonary bypass reduces myocardial injury. Perfusion 2001;16:43–49.

43 Sheppard SV: Are the occasional high pressure gradients associated with leucocyte-reducing filters and oxygenators a similar phenomenon? Perfusionist 2000;24(3):7–10.
44 Sheppard SV, Huxtable S, Smith DC, Gibbs RV: Does the use of aggressive blood cooling during CPB cause fibrin precipitation? 25th Anniversary Congress on Perfusion, Jersey, 1999.
45 Fukushima N, Shirakura R, Nakata S, Kanejo M, Sawa Y, Matsuwaka R, Naka Y, Matsumiya G, Matsuda H: Effects of terminal cardioplegia with leukocyte depleted blood on heart grafts preserved for 24 hours. J Heart Lung Transplant 1992;11:676–682.
46 Kawata H, Sawatari K, Mayer JE: Evidence for the role of neutrophils in reperfusion injury after cold cardioplegic ischemia in neonatal lambs. J Thorac Cardiovasc Surg 1992;103:908–918.
47 Breda MA, Drinkwater DC, Laks H, Bhuta S, Corno AF, Davtyan HD, Chang P: Prevention of reperfusion injury in the neonatal heart with leukocyte depleted blood. J Thorac Cardiovasc Surg 1989;97:654–665.
48 Pearl JM, Drinkwater DC, Laks H, Stein DG, Capouya ER, Bhuta S: Leucocyte-depleted reperfusion of transplanted human hearts prevents ultrastructural evidence of reperfusion injury. J Surg Res 1992;52:298–308.
49 Ichihara T, Yasuura K, Maseki T, et al: The effects of using a leucocyte removal filter during cold blood cardioplegia. Surg Today Jap J Surg 1994;24:966–972.
50 Pala MG, Paolini G, Paroni R, De Vecchi E, Gallorini C, Stefano PL, Grossi A: Myocardial protection with and without leucocyte depletion: A comparative study on the oxidative stress. Proc Ann Meet Eur Assoc Cardiothorac Surg, The Hague, September 1994.
51 Palatainos G, Balentine G: Leucocyte filtered blood cardioplegia and controlled reperfusion. International congress of the society of cardiovascular surgery, Berlin, 1994.
52 Sawa Y, Matsuda H, Shimazaki Y: Evaluation of leucocyte depleted terminal blood cardioplegia in patients undergoing elective and emergency coronary bypass grafting. J Thorac Cardiovasc Surg 1994;108:1125–1131.
53 De Vecchi E, Paroni R, Pala MG: Role of leucocytes in free radical production during myocardial revascularisation. Heart 1997;77:449–455.
54 Palatianos GM, Balentine G, Astras G, Prapas S, Triantafillou C, et al: Leukocyte filtration reduces myocardial reperfusion morbidity. STS, Fort Lauderdale, 2000.
55 Roth M: Single leucocyte filter (Pall BC1B) fails in multidose cold blood cardioplegia. J Thorac Cardiovasc Surg 1997;113:1116–1117.
56 Heggie A, Corder JS, Crichton PR, Hesford JW, Bingham H, Jeffries S, Hooper TL: Clinical evaluation of the new Pall leucocyte depleting blood cardioplegia filter (BC1). Perfusion 1998;13: 17–25.
57 Suzuki I, Ogoshi N, Chiba M: Clinical evaluation of a leucocyte-depleting blood cardioplegia filter (BC1B) for elective open-heart surgery. Perfusion 1998;13:205–210.
58 Nakamae K: Experimental use of the blood cardioplegia filter BC1B. J Extracorp Tech 1998; 24(3):70–75.
59 Roth M, Kraus B, Scheffold T, Reuthebuch O, Klövekorn WP, Bauer EP: The effect of leukocyte-depleted blood cardioplegia in patients with severe left ventricular dysfunction: A randomized, double-blind study. Thorac Cardiovasc Surg 2000;120:642–650.
60 Browning PG, Pullan M, Jackson M: Leucocyte-depleted cardioplegia does not reduce reperfusion injury in hypothermic coronary artery bypass surgery. Perfusion 1999;14:371–377.
61 Matsuda H, Fukushima N, Sawa Y: First brain dead donor heart transplantation under new legislation in Japan. Jpn J Thorac Cardiovasc Surg 1999;47:499–505.
62 Hayashi Y, Sawa Y, Nishimura M: Clinical evaluation of leukocyte-depleted blood cardioplegia for pediatric open heart operation. Ann Thorac Surg 2000;69:1914–1919.
63 Hansboro S: Clinical evaluation of the Pall BC1B leucocyte depleting blood cardioplegia filter. Therapeutic Applications of Leukocyte Filtration Workshop, London, 2000.

Dr. Stuart V. Sheppard, Southampton University Hospital,
Tremona Road, Southampton SO16 6Y0 (UK)
Tel. +44 23 8079 6930, Fax +44 23 8079 4037, E-Mail stuart.sheppard@suht.swest.nhs.uk

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 33–43

Transfusion: Pathology and Relevance of Leukocyte Reduction

Reinhard Henschler

Department of Cell Production and Stem Cell Biology Group, Institute for Transfusion Medicine and Immune Hematology, German Red Cross Blood Donation Center, Frankfurt, Germany

Overview: The Spectrum of Side Effects after Transfusion of Blood Components

The most common side effects of transfusion of blood products are of an immunological nature. Immune-mediated reactions are observed in up to 37.5% of transfused patients, notably in patients receiving platelet concentrates who develop febrile reactions [1], yet life-threatening complications occur at much lower frequencies. In contrast, the frequency of non-immune-mediated transfusion reactions, mostly due to transmission of viruses, has decreased to levels far below 1 in 100,000 transfusion events with the use of a sophisticated and highly efficient multistage system of laboratory tests including serological and molecular biological techniques [2, 3]. A summary of pathological reactions to transfused blood components is given in table 1. This chapter summarizes essential data and immunomechanisms leading to pathological reactions to transfusion of blood.

Hemolytic Reactions

Acute hemolysis due to blood group antigen incompatibility is the most common cause of death after blood transfusion. The key event is the reaction of alloantibodies with their respective antigens on erythrocytes. If the antibodies are present in the recipient serum already at the time of transfusion, an immediate reaction can occur; however, protracted courses with later onset are seen if the recipient's immune system has not been sensitized to the antigen previously. Red cell lysis can be due to two types of attack by immune effector cells: (1) full complement activation beyond the level of C3b, resulting in intravasal lysis, or (2) complement activation only up to the level of C3, generally resulting in

Table 1. Mechanisms of immune-mediated adverse transfusion effects

Classification of reaction	Mediators
Hemolytic transfusion reactions	recipient IgG against erythrocytes, complement components
Febrile nonhemolytic transfusion reactions	proinflammatory cytokines released from donor leukocytes or host antileukocyte antibodies
Posttransfusion purpura	host antiplatelet antibodies
Transfusion-induced acute lung injury	host antigranulocyte or anti-HLA antibodies
Allergic reactions	host antibodies against soluble antigens
Transfusion-induced graft-versus-host disease	donor lymphocytes

extravasal lysis. In this case, macrophages, especially Kupffer cells in the liver which carry C3b receptors, sequestrate the opsonized erythrocytes. In addition, a second signal which will potentiate the response can be elicited by IgG through Fc-γ receptors on phagocytes [4].

The response in patients involves activation of mast cells via complement degradation products C4a, C3a, C5a, with subsequent systemic histamine and serotonin release. This results in hypotonia and shock, and especially, in renal perfusion injury. Interestingly, in contrast to hemoglobin-free red cell fragments [5], infusion of free hemoglobin did not result in nephrotoxicity. As an additional mechanism, complement activation after ABO-incompatible transfusion of red cells has been shown to result from increased levels of proinflammatory cytokines, such as IL-8 and TNF-α [6, 7]. Very likely, the reaction upon TNF-α involves the stimulation of tissue factor expression on endothelial cells and monocytes [8], leading to disseminated intravasal coagulation [9].

The therapy of a patient after a hemolytic transfusion reaction is mainly based on measures to counteract the shock syndrome, renal failure, and the sequelae of disseminated intravasal coagulation.

Febrile Nonhemolytic Transfusion Reactions

By definition, febrile nonhemolytic transfusion reactions (FNHTR) are transfusion-associated reactions with an increase in body temperature of ⩾1 °C, in the absence of other symptoms or signs of hemolytic reactions. FNHTR can be caused by different mechanisms (fig. 1): firstly, transfused leukocytes which are reactive against host cells are stimulated to release cytokines [10]. Secondly, alloantibodies directed against transfused leukocytes and platelets present in the host [11]. Thirdly, a correlation has been established between the levels of leukocytes, the storage time and the levels of proinflammatory cytokines, such as IL-1β, TNF-α, IL-6 and IL-8 [1, 12, 13], indicating a direct role of preformed, transfused

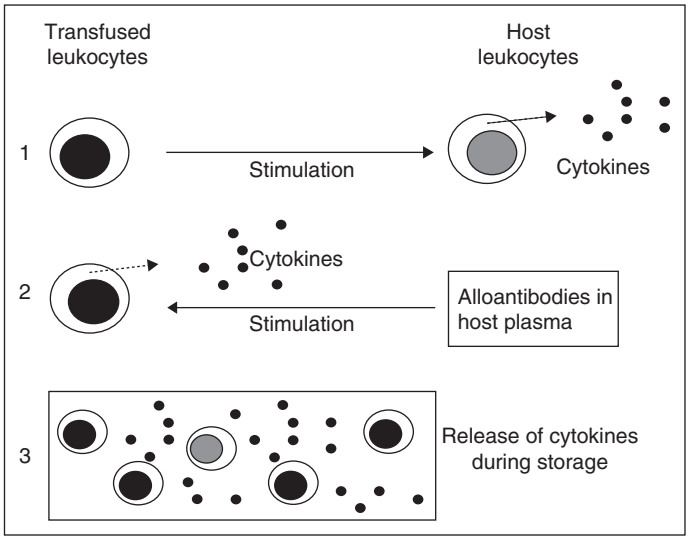


Fig. 1. Pathomechanisms of FNHTR. Donor-derived leukocytes may act either after infusion within the host (1, 2), or before transfusion during storage (3). Cytokines are released either from host leukocytes after allogeneic stimulation by transfused leukocytes (1), by the transfused leukocytes after stimulation by alloantibodies which are present in the host plasma (2), or by release of cytokines during storage before transfusion.

cytokines in the elicitation of the fever response. The fever response itself has been proposed to result from the increased serum levels of cytokines and the integrated stimulation of phagocyte activation via the TNF and IL-1 pathway [14].

Typically, FNHTR are seen within 30 min–2 h after starting the transfusion. Subjectively, patients feel seriously ill, yet the prognosis is good. However, as a fever could also be the first sign of a hemolytic reaction or a reaction to bacterially contaminated material, these events have to be excluded before diagnosing FNHTR. The therapy consists of immediate arrest of the transfusion and antipyretic pharmacotherapy.

Posttransfusion Purpura

Purpura is the sign of platelet insufficiency; accordingly, specific antiplatelet alloantibodies have been detected in patients developing this disease; these alloantibodies can be directed against a class of human platelet-specific antigens (HPA antigens) [15]. There may also be a genetic predisposition associated with the expression of HLA-DR3, or DRw52a [16, 17]. Posttransfusion purpura occurs only rarely, and with a delay of 5–10 days after the transfusion of platelet concentrates. However, the mortality is relatively high (10–20%).

Intravenous IgG has been found to be a potent drug that positively influences the course of the disease [18].

Transfusion-Associated Acute Lung Injury

Transfusion-associated acute lung injury (TRALI) is caused by the reaction of alloantibodies directed against granulocytes or anti-HLA antibodies which are present in the host [19]. As a consequence, granulocytes become activated and lung injury results from the penetration of the activated granulocytes into alveolar epithelia [20]. Again, local release of cytokines and chemokines, as well as of other products of the activated cells, has been described as a causal principle for the pulmonary symptoms which, in combination with fever, prevail clinically [21]. The symptoms typically occur within 2–6 h after starting the transfusion, and require intubation and subsequent intensive care in most cases [22, 23].

Although TRALI is rare, it accounted for 355 transfusion-associated deaths in the US over a period of 10 years and is the second most common cause of death after transfusion [24].

Transfusion-Induced Graft-versus-Host Disease

Transfusion-induced graft-versus-host disease (TI-GVHD) is one of the most serious complications of blood transfusion. It arises from a reaction of the donor's lymphocytes with recipient cells in a situation of compromised recipient lymphocytes which could eliminate the donor cells [25]. However, TI-GVHD was also shown to occur in immunocompetent recipients, in the case of transfusion of blood from related donors. These patients mostly suffer from underlying diseases, such as Hodgkin's disease, non-Hodgkin lymphomas, leukemias or congenital immunodeficiencies.

The critical threshold cell dose for induction of TI-GVHD is relatively high ($\geqslant 10^7$/kg body weight) [26]. The pathogenesis of TI-GVHD depends on the ability of the transfused lymphocytes to recognize allogeneic HLA class I or class II antigens, and to proliferate in the recipient. In the course of the disease, however, the involvement of other cells, especially mononuclear cell populations and the production of relatively large amounts of cytokines have been implicated [27]. Clinically, patients display a fever, dermatitis, hepatitis, diarrhea, bone marrow aplasia and infections. The diagnosis of TI-GVHD is confirmed by the detection of donor lymphocytes in the host by genetic markers, including DNA fingerprinting or the polymerase chain reaction [28]. The occurrence of TI-GVHD is prevented by gamma-irradiation of all blood components with 30 Gy. In addition, the general vigorous depletion of leukocytes by the inline filtration technique (see below) may also have the potential to prevent TI-GVHD.

Indications for irradiation of blood components include patients in the course of allogenic or autologous bone marrow or blood stem cell transplantation,

cases of congenital immunodeficiencies, decreased T lymphocyte function, intrauterine transfusion and transfusion of newborns, patients with hematopoietic malignancies, previous blood or organ donation to related individuals, and recipients or potential recipients of HLA-typed blood components [29].

Allergic Reactions

Allergic reactions can result from antibodies present in the recipient of blood components, and directed against soluble components of plasma. The allergens can be bound to plasma proteins, e.g. albumin. There are two types of allergic reactions [30]. (1) Anaphylactic reactions: many of these cases exhibit an IgA deficiency associated with high levels of anti-IgA antibodies. The transfusion has to be stopped immediately and followed by the injection of, e.g., epinephrine or steroids. These patients must receive IgA-free blood products. (2) Urticaria: this type of reaction is very common. Typically, there is no fever. The transfusion should be delayed until after the administration of an antihistaminic drug and the resolution of the symptoms.

Non-Immunologically-Mediated Adverse Effects: Virus Transmission (HBV, HCV, HIV, CMV)

Contamination of blood products by hepatitis viruses and the HIV have been the major cause for transfusion-induced morbidity from infectious agents. Especially, the administration of pooled plasma products to the cohort of patients with acquired coagulation factor deficiencies has caused hundreds and thousands of infections and transfusion-related deaths in different areas of the world. The clinical course of transfusion-transmitted HBV or HCV or HIV infection generally resembles that of infections acquired by other routes, e.g. sexual transmission.

The testing of blood products has since undergone major improvements. Today, serology combined with the nucleic amplification testing (NAT) technology allows, if handled correctly, to practically completely exclude products harboring HBV, HCV and HIV [31]. Very recently, the risk for a given blood product to contain HCV has been reported to range between 1 : 185,000 and 1:308,000 [3]. In a nationwide study initiated by our institution, NAT was able to detect 1 HBV-positive sample in about 2,000,000 donations tested, 1 HCV-positive sample in about 1,000,000 and 1 HIV-positive sample in about 340,000 seronegative donors [2]. Both studies were based on the examination of more than 5 million individual donations.

CMV Infection. In contrast to the above-mentioned viruses, CMV resides in leukocytes and is therefore primarily transmitted by leukocyte-containing cell preparations, i.e. platelet concentrates and red cell concentrates. Otherwise the virus is not highly contagious, making transfusion the major cause of CMV transmission. The prerequisite for severe CMV infection is an immunocompromised

host, as induced by pharmacological immunosuppression and the bone marrow aplasia following bone marrow transplantation. The major clinical problem resulting from CMV infection is an interstitial pneumonia, which is reponsible for most CMV-related deaths. The delivery of blood products testing negative for anti-CMV antibodies reduces the incidence of CMV transmission to these recipients to 1–4% instead of 28–57% [32, 33]. The possibility to almost completely eliminate leukocytes via inline leukocyte filtration (see below) has led to the general introduction of leukocyte-filtered blood products for this category of blood transfusion recipients (see below 'CMV Transmission').

Other Side Effects

Other, less frequent side effects of blood component transfusion include bacterial contamination (nearly exclusively in platelet concentrates, as these are stored at room temperature), hypervolemia in the recipient, embolism, especially by trapped air or microaggregates which are not retained by the standard large-mesh (170–200 μm) transfusion filters, transfusion-associated hemosiderosis, citrate and sodium intoxication, or suspected toxicities by constituents of plastic bags which may dissolve at low concentrations and reach the recipient. Overall, they remain of borderline relevance, however.

Principles of Leukocyte Depletion of Blood Products

Various technologies have been developed to eliminate leukocytes from blood components. The blood component separation procedure, termed 'buffy coat removal' method, which is used throughout most of Europe, results in a much better depletion of leukocytes from red cell concentrates (over 90%) as compared with the methodology generally used in North America [34]. However, various additional filter materials, such as cotton wool and synthetic mesh materials, have been developed to retain leukocytes by adhesion. One general principle was to induce platelet activation and to utilize the upregulation of adhesion avidity on the activated platelets to subsequently and quantitatively capture leukocytes [for a review, see Dzik, 10]. Many such filters have been developed primarily for use at the bedside. They are easy to connect into the blood transfusion tubing systems, and the term 'bedside filters' has become commonly used for these systems. Up to now, they have generally been recommended for leukocyte reduction in a number of indications, yet their efficacy and reproducibility have not been demonstrated conclusively, or have been found to be inadequate in some instances [35, 36].

New generations of more effective leukocyte filters, operated under Good Manufacturing Practice conditions, have allowed to reach leukocyte levels of

below 5×10^6 per unit (blood bank filters [10]). These have become mandatory in all cases where a maximum leukocyte depletion is required, e.g. after transplantation to avoid host-versus-graft reactions. These systems, termed 'blood bank leukocyte filters' have only recently been outdated by even more advanced filters, which are now integrated into closed-circuit blood collection and separation systems, and are called 'inline leukocyte depletion filters'. The technology allows 'prestorage' removal of leukocytes already at the time of blood component separation, i.e. within the first 24 h after blood collection. These filters deplete leukocytes by 99.9–99.99%, thus allowing general leukocyte levels of below 1×10^6 per unit. Such levels can now be reached in routine production of blood products and are considered suitable for the prevention of transfusion-related effects due to transmitted leukocytes [10].

Proven Positive Effects of Leukocyte Depletion

A number of clinical studies have been conducted in the last 10 years on the first generations of leukocyte filters in order to delineate the benefits of leukocyte depletion in various patient groups.

Febrile Nonhemolytic Transfusion Reactions. The incidence of FNHTR after transfusion of red cell and platelet concentrates in cancer patients was found to be 6.7 and 37.5%, respectively. Bedside filters have been sufficient to eliminate virtually all FNHTR after transfusion of red cell concentrates, yet after platelet concentrates, only a partial reduction was seen [37, 38]. This is explained in figure 1. The levels of proinflammatory soluble mediators, such as IL-1β, IL-6, TNF-α and IL-8 have been shown to dramatically increase during storage of platelet concentrates [12]. On average, a 300-ml pool of platelet concentrate can contain 100 ng of IL-6 or 20 ng of TNF-α. By mimicking endogenous levels of these mediators that are reached systemically, e.g. during endotoxinemia, it was calculated that these amounts of cytokines suffice to induce severe fever reactions. The introduction of inline (prestorage) leukocyte depletion results in platelet concentrates that are virtually free of these mediators [12], and is expected to lead to a strong decrease of FNHTR, in excess of the effects observed so far with previous filter generations.

HLA Sensitization. Various endpoints have been used to detect the induction of anti-HLA responsiveness in recipients of blood transfusions: anti-HLA antibodies in the serum are used by most investigators, but of course this cannot measure the clinical impact of the tested antibodies. Therefore, e.g., the refractoriness to platelet transfusions, the number of platelet transfusions required in a given time frame and the interval between transfusions have been introduced as clinically relevant endpoints for demonstrating the presence of transfusion-induced anti-HLA antibodies. In addition to a lack of clarity in this respect, anti-HLA reactivity present before a blood transfusion has to be taken into

account. After transfusion of platelet concentrates it has been found that although the rate of anti-HLA antibodies decreased after bedside filtration of platelet concentrates, the number of patients developing platelet refractoriness thought to be the consequence of the sensitization did not decrease significantly in a cohort of patients with hematological malignancies [39]. A Canadian multicentric study [40], as well as a number of nonrandomized single-center studies [41, 42] showed that use of inline leukocyte-depleted platelet concentrates diminishes the rate of patients developing platelet refractoriness by more than half compared with nonfiltered products from random donors, making this product equivalent to apheresis-derived platelets. Therefore, the reduction of the anti-HLA response has broad clinical relevance in patients receiving filtered platelet concentrates.

The situation is not so clear in the case of red cell concentrates and surgical patients. Especially, the mechanisms of 'immunomodulation', as reflected by the term itself, have been rather elusive. However, postoperative complications, including pneumonia and death, as well as reduced durations of hospital stays, have been found to be decreased in patients undergoing gastrointestinal surgery [43]. However, more investigations will be necessary to better understand the reasons for these effects.

CMV Transmission. Random platelet concentrates result in an up to 4% transmission of CMV despite testing [44]. Also, latent CMV can be activated by allogeneic leukocytes from CMV-seronegative donors. Clinical studies have been conducted to confirm the role of leukocyte depletion in CMV transmission. The incidence of CMV transmission was similar in patients receiving random non-CMV-tested prestorage-filtered platelet concentrates and in patients receiving random platelets not leukodepleted before storage, but tested for the presence of anti-CMV antibodies [45].

It has been shown in vitro that leukocytes that harbor quiescent CMV may be stimulated to reactivate the virus [46] and that these leukocytes can transfer CMV to endothelial cells and vice versa [47]. A growing body of evidence exists that persistent CMV may also manipulate the cell-mediated immune system and thus may contribute to the generation of a variety of immune-mediated disorders also in the absence of measurable virus replication [48].

Impact of Therapy with Leukocyte-Depleted Blood Products in Patients Undergoing Cardiac Surgery

Van de Watering et al. [49] conducted a randomized clinical trial on 914 patients undergoing cardiovascular bypass or valve replacement surgery who all received red cell concentrates. The patients were divided into three groups: one

group received buffy-coat-reduced products (high leukocyte levels, i.e. about 10^9 leukocytes/unit); the second group received blood-blank-filtered concentrates (5×10^6 leukocytes/unit) and the third group received prestorage inline-filtered concentrates (1×10^6 leukocytes/unit). 195 postoperative bacterial infections occurred in 175 patients. The difference in mortality due to noncardiac causes between the group receiving nonfiltered concentrates and the two groups receiving filtered concentrates is statistically significant ($p < 0.015$) and demonstrates a significant advantage of leukocyte reduction in this patient cohort in terms of survival. The reason for this finding is unclear. Together with data from other surgical patient groups (see above, 'HLA Sensitization'), these observations warrant further intensive investigations to resolve the underlying mechanisms.

References

1 Heddle N, Klama L, et al: A prospective study to identify the risk factors associated with acute reactions to platelet and red cell transfusion. Transfusion 1993;33:794–797.
2 Seifried E, Faber R, et al: HCV, HBV and HIV screening in 7 million blood donors – Efficacy and economical results (abstract no 241). Blood 2000;96(suppl 1):58a.
3 Stramer SL, Caglioti S, Strong DM: NAT of the United States and Canadian blood supply. Transfusion 2001;40:1165–1167.
4 Horsewood P, Kelton JD: Macrophage-mediated cell destruction; in Garratty G (ed): Immunobiology of Transfusion Medicine. New York, Dekker, 1994, pp 435–464.
5 Rabiner S, O'Brien K, et al: Further studies with stroma-free hemoglobin solution. Ann Surg 1970;171:615–622.
6 Davenport R, Strieter R, et al: Interleukin-8 production in red blood cell incompatibility. Blood 1990;76:2439–2442.
7 Davenport R, Strieter R, et al: Red cell ABO incompatibility and production of tumor necrosis factor alpha. Br J Haematol 1991;78:540–544.
8 Conkling P, Greenberg C, et al: Tumor necrosis factor induces tissue-factor-like activity in human leukemic cell line U 937 and peripheral blood monocytes. Blood 1988;72:128–133.
9 Davenport R, Burdick M, et al: Cytokine production in IgG-mediated red cell incompatibility. Transfusion 1993;33:19–24.
10 Dzik S, Aubuchon J, Jeffries L, Kleinman S, Manno C, Murphy MF, Popovsky MA, Sayers M, Silberstein LE, Slichter SJ, Vamvakas EC: Leukocyte reduction of blood components: Public policy and new technology. Transfus Med Rev 2000;14:34–52.
11 Decary F, Ferner P, et al: An investigation of non-haemolytic transfusion reactions. Vox Sang 1984;46:277–285.
12 Aye M, Palmer D, et al: Effect of filtration of platelet concentrates on the accumulation of cytokines and platelet release factors during storage. Transfusion 1995;35:117–124.
13 Muylle L, Joos M, Wouters E, De Bock R, Peetermans ME: Increased tumor necrosis factor alpha (TNFalpha), interleukin 1, and interleukin 6 (IL-6) levels in the plasma of stored platelet concentrates: Relationship between TNFalpha and IL-6 levels and febrile transfusion reactions. Transfusion 1993;33:195–199.
14 Dinarello CA, Cannon JG, Wolff SM, Bernheim HA, Beutler B, Cerami A, Figari IS, Palladino MA Jr, O'Connor JV: Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. J Exp Med 1986;163:1433–1450.
15 von dem Borne A, Simsek S, et al: Platelet and neutrophil alloantigens: Their nature and role of immune-mediated cytopenias; in Garratty G (ed): Immunobiology of Transfusion Medicine. New York, Dekker, 1994, pp 149–171.

16 Mueller-Eckhardt C, Mueller-Eckhardt G, et al: Immunogenicity of and immune response to the human platelet antigen Zwa is strongly associated with HLA-B8 and DR3. Tissue Antigens 1985; 26:71–76.
17 Decary F, L'Abbe D, et al: The immune response to the HPA-1a antigen: Association with HLA-DRw 52a. Transfusion 1991;1:55–62.
18 Mueller-Eckhardt C, Kiefel V: High dose IgG for posttransfusion purpura revisited. Blut 1988; 570:163–167.
19 Eastlund T, Mc Grath P, et al: Fatal pulmonary transfusion reaction to plasma containing donor HLA antibody. Vox Sang 1989;57:63–66.
20 Seeger W, Schneider U, et al: Reproduction of transfusion-related acute lung injury in an ex vivo lung model. Blood 1990;76:1438–1444.
21 Mollison PL, Engelfriet CP, Contreras M: Blood Transfusion in Clinical Medicine. Oxford, Blackwell Science 1993.
22 Popovsky M, Moore S: Diagnostic and pathogenetic considerations in transfusion-related acute lung injury. Transfusion 1985;25:573–577.
23 Popovsky M, Chaplin H, et al: Transfusion related acute lung injury. A neglected, serious complication of hemotherapy. Transfusion 1992;32:589–592.
24 Sazama K: Reports of 355 transfusion-associated deaths: 1976 through 1985. Transfusion 1990;30:583–590.
25 Sazama K, Holland P: Transfusion-induced graft-versus-host disease; in Garratty G (ed): Immunobiology of Transfusion Medicine. New York, Dekker, 1994, pp 631–656.
26 Leitman S: Use of blood cell irradiation in the prevention of posttransfusion graft-vs-host disease. Transfus Sci 1989;10:219–232.
27 Ferrara L: The febrile platelet transfusion reaction: A cytokine shower. Transfusion 1995;35: 89–90.
28 Kunstmann E, Bocker T, et al: Diagnosis of transfusion-associated graft versus host disease by genetic fingerprinting and polymerase chain reaction. Transfusion 1992;32:766–770.
29 Linden JV, Pisciotto PT: Transfusion-associated graft-versus-host disease and blood irradiation. Transfus Med Rev 1992;6:116–123.
30 Sandler SG, Mallory D, Malamut D, Eckrich R: IgA anaphylactic transfusion reactions. Transfus Med Rev 1995;9:1–8.
31 Busch M, Stramer S, et al: Evolving applications of nucleic acid amplication assay for prevention of virus transmission by blood components and derivates; in Garratty G (ed): Applications of Molecular Biology to Blood Transfusion Medicine. Bethesda, American Association of Blood Banks, 1997, vol 123, p 76.
32 Meyers J, Flournoy N, et al: Risk factors for cytomegalovirus infection after human marrow transplantation. J Infect Dis 1986;153:478.
33 Bowden R, Silichter S, et al: Comparison of filtered leukocyte-reduced and cytomegalovirus (CMV) seronegative blood products for the prevention of transfusion-associated CMV infection after marrow transplant. Blood 1995;86:3598–3603.
34 McCullough MD: Preparation, storage, and characteristics of blood components and plasma derivatives; in Transfusion Medecine. New York, McGraw-Hill, pp 67–96.
35 Ledent E, Berlin G: Inadequate white cell reduction by bedside filtration of red cell concentrates. Transfusion 1994;34:765–768.
36 Sprogoe-Jakobsen U, Saetre AM, Georgsen J: Preparation of white cell-reduced red cells by filtration: Comparison of a bedside filter and two blood bank filter systems. Transfusion 1995;35: 421–426.
37 Goodnough LT, Riddell J, Lazarus H, Chafel TL, Prince G, Hendrix D, Yomtovian R: Prevalence of platelet transfusion reactions before and after implementation of leukocyte depleted platelet concentrates by filtration. Vox Sang 1993;65:103–107.
38 Heddle N, Klama L, et al: The role of the plasma from platelet concentrates in transfusion reactions. N Engl J Med 1994;331:625–628.
39 Williamson LM, Wimperis JF, et al: Bedside filtration of blood products in the prevention of HLA alluminization – a prospective randomized study. Blood 1994;83:3028–3035

40 The Trial to Reduce Alloimmunization to Platelets Study Group. Leukocyte reduction and ultraviolet B irradiation od platelets to prevent alloimmunization and refractoriness to platelet transfusion. N Engl J Med 1997;337:1861–1869.
41 Murphy M, Metcalfe P, et al: Use of leucocyte-poor blood components and HLA-matched-platelet donors to prevent HLA alloimmunization. Br J Haematol 1986;62:529–534.
42 Oksanen K, Kekomaki R, et al: Prevention of alloimmunization in patients with acute leukemia by use of white cell-reduced blood components – a randomized trial. Transfusion 1991;31:588–594.
43 Jensen LS, Kissmeyer-Nielsen P, Wolff B, Qvist N: Randomised comparison of leucocyte-depleted versus buffy-coat-poor blood transfusion and complications after colorectal surgery. Lancet 1996;348:841–845.
44 Bowden R, Sayers M, et al: Cytomegalovirus immune globulin and seronegative blood products to prevent primary cytomegalovirus infection after marrow transplantation. N Engl J Med 1986; 314:1006.
45 Larsson S, Soderberg-Naucler C, Wang FZ, Moller E: Cytomegalovirus DNA can be detected in peripheral blood mononuclear cells from all seropositive and most seronegative healthy blood donors over time. Transfusion 1998;38:271–278.
46 Soderberg-Naucler C, Fish KN, Nelson JA: Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. Cell 1997;91:119–126.
47 Waldman WJ, et al: Bidirectional transmission of infectious cytomegalovirus between monocytes and vascular endothelial cells: An in vitro model. J Infect Dis 1995;171:263–272.
48 Scholz M, et al: Cytomegalovirus, oxidative stress and inflammation as interdependent pathomechanisms: Need for novel therapeutic strategies? in Scholz M, Rabenau HF, Doerr HW, Cinatl J (eds): CMV-related immunopathology. Monogr Virol. Basel, Karger, 1998, vol 21, pp 90–105.
49 Van de Watering L, Hermans J, et al: Beneficial effects of leukocyte depletion of transfused blood on postoperative complications in patients undergoing cardiac surgery: A randomized clinical trial. Circulation 1998;97:562–568.

Dr. Reinhard Henschler, Head, Department of Cell Production and Stem Cell Biology Group,
Institute for Transfusion Medicine and Immune Hematology,
German Red Cross Blood Donation Center, Sandhofstrasse 1, D–60528 Frankfurt (Germany)
Tel. +49 69 6782 191/242, Fax +49 69 6782 258, E-Mail rhenschler@bsdhessen.de

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 44–46

Immunopathogenicity in Heart, Heart-Lung Transplantation: Relevance of Leukocyte Depletion

Martin Scholz, Georg Matheis

Department of Thoracic and Cardiovascular Surgery, J.W. Goethe University, Frankfurt, Germany

Leukocyte-Mediated Immunopathogenicity in Heart, Heart-Lung Transplantation

The major problems related to organ transplantation are (1) the recognition of allogeneic tissue by T cells leading to graft rejection (host-versus-graft reaction) and (2) postoperative bacterial and/or viral infection. Stress, allogenicity and infection seem to be interdependent pathogenic factors that cannot be regarded as isolated pathomechanisms [1]. In humans, HLA typing allows the matching between recipient and donor in order to prevent acute or hyperacute rejection of the grafts. Leukocyte depletion, of course, cannot prevent the allogeneic immune responses directed against the non-self tissue but has been shown to have beneficial effects, e.g. when applied during reperfusion of the preserved transplant.

Experimental Evaluation of Leukocyte Depletion in Heart, Heart-Lung Transplantation

Leukocyte sequestration within the lung seems to be the major pathogenic effect upon lung preservation and reperfusion in the context of heart-lung transplantation. In this regard, some experimental studies that were done to evaluate the effects of leukocyte depletion of the reperfused blood following prolonged

Table 1. Leukocyte depletion in animal heart and lung transplantation models

Reference	Study results
Bando et al. [6]	Improved pulmonary function, reduced free radical generation
Bando et al. [13]	Less extravascular lung water, lower PVR, higher PaO_2
Breda et al. [2]	24-h lung preservation
Breda et al. [11]	Myocardial damage reduced and work performance retained after 12 h of preservation
Byrne et al. [15]	Better myocardial blood flow and LV stroke work index
Fukushima et al. [8]	Increase in recruitable cardiac output and stroke work in heart grafts preserved for 24 h
Hall et al. [10]	Reduced injury to the lung after hypothermic ischemia
Kawata et al. [14]	Improved recovery of endothelial and mechanical function
Pillai et al. [3]	Less extravascular lung water and higher PaO_2
Ross et al. [9]	Reduced neutrophil infiltration
Schueler et al. [4, 12]	Improvement in extravascular lung water, airway pressure, PaO_2, PAP, PVR
Shiraishi et al. [5]	Reduced lung injury after 10-min leukocyte depletion
Stein et al. [7]	Complete functional recovery with aspartate/glutamate-enriched leukocyte-depleted blood

lung preservation showed beneficial effects in terms of parameters such as systemic arterial oxygenation, pulmonary artery pressure, lung water content, histology [2–4]. A 10-min leukocyte depletion during reperfusion after rewarming ischemia was sufficient to obtain beneficial results in reducing lung injury in neonatal piglet heart-lung blocks [5]. Moreover, leukocyte depletion reduced cardiopulmonary bypass (CPB)-associated lung injury resulting from oxygen radicals [6]. Stein et al. [7] reported on a beneficial effect of a combination of aspartate/glutamate-enriched leukocyte-depleted blood cardioplegia when neonatal piglet hearts were reperfused after 24 h of preservation with University of Wisconsin solution. However, leukocyte depletion alone had no significant effect. In contrast, canine heart graft preservation for 24 h in Collins solution with terminal warm-blood cardioplegia with leukocyte depletion has been reported to improve the preload recruitable stroke work after both heterotopic and orthotopic transplantation [8]. Reduced neutrophil infiltration and microvascular permeability (detected with Evan's blue) have been achieved in ischemic lungs by using leukocyte-depleted blood reperfusate [9]. These and other results [10–15] are summarized in table 1 (modified from Palanzo [16] in this book).

References

1 Scholz M, et al: Cytomegalovirus, oxidative stress and inflammation as interdependent pathomechanisms: Need for novel therapeutic strategies?; in Scholz M, Rabenau HF, Doerr HW, Cinatl J (eds): CMV-Related Immunopathology. Monogr Virol. Basel, Karger, 1998, vol 21, pp 90–105.

2 Breda MA, Hall TS, Stuart RS, Baumgartner WA, Borkon AM, Brawn JD, Hutchins GM, Reitz BA: Twenty-four hour lung preservation by hypothermia and leukocyte depletion. J Heart Transplant 1985;4:325–329.

3 Pillai R, Bando K, Schueler S, Zebly M, Reitz BA, Baumgartner WA: Leukocyte depletion results in excellent heart-lung function after 12 hours of storage. Ann Thorac Surg 1990;50:211–214.

4 Schueler S, De Valeria PA, Hatanaka M, Cameron DE, Bando K, Zeebley M, Hutchins GM, Reitz BA, Baumgartner BA: Successful twenty-four hour lung preservation with donor core cooling and leukocyte depletion in an orthotopic double lung transplantation model. J Thorac Cardiovasc Surg 1992;104:73–82.

5 Shiraishi Y, Lee JR, Laks H, Waters PF, Meneshian A, Marelli D, Blitz A, Chang P: Use of leukocyte depletion to decrease injury after lung preservation and rewarming ischemia: An experimental model. J Heart Lung Transplant 1998;17:250–258.

6 Bando K, Pillai R, Cameron DE, et al: Leukocyte depletion ameliorates free-radical-mediated lung injury after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;99:873–877.

7 Stein DG, Permut LC, Drinkwater DC Jr, Bhuta S, Chang P, Wu A, Laks H: Complete functional recovery after 24-hour heart preservation with University of Wisconsin solution and modified reperfusion. Circulation 1991;84:III316–III323.

8 Fukushima N, Shirakura R, Nakata S, Kaneko M, Sawa Y, Matsuwaka R, Naka Y, Matsumiya G, Matsuda H: Effects of terminal cardioplegia with leukocyte-depleted blood on heart grafts preserved for 24 hours. J Heart Lung Transplant 1992;11:676–682.

9 Ross SD, Tribble CG, Gaughen JR Jr, Shockey KS, Parrino PE, Kron IL: Reduced neutrophil infiltration protects against lung reperfusion injury after transplantation. Ann Thorac Surg 1999;67:1428–1433.

10 Hall TS, Breda MA, Baumgartner WA, et al: The role of leukocyte depletion in reducing injury to the lung after hypothermic ischemia. Curr Surg 1987;44:137–139.

11 Breda MA, Drinkwater DC, Laks H, et al: Prevention of reperfusion injury in the neonatal heart with leukocyte-depleted blood. J Thorac Cardiovasc Surg 1989;97:654–665.

12 Schueler S, Hatanaka M, Bando K, et al: Twenty-four hour lung preservation with donor core-cooling and leukocyte depletion in a bilateral lung transplant model. Surg Forum 1990;41:405–407.

13 Bando K, Scheuler S, Cameron DE, et al: Twelve-hour cardiopulmonary preservation using donor core-cooling, leukocyte depletion and liposomal superoxide dismutase. J Heart Lung Transplant 1991;10:304–309.

14 Kawata H, Sawatari K, Mayer JE: Evidence for the role of neutrophils in reperfusion injury after cold cardioplegic ischemia in neonatal lambs. J Thorac Cardiovasc Surg 1992;103:908–918.

15 Byrne JG, Appleyard RF, Lee CC, et al: Controlled reperfusion of the regionally ischemic myocardium with leukocyte-depleted blood. J Thorac Cardiovasc Surg 1992;103:66–72.

16 Palanzo DA: Leukocyte filters in perfusion; in Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology. Basel, Karger, 2002, pp 89–100.

PD Dr. Martin Scholz, Department of Thoracic and Cardiovascular Surgery,
J.W. Goethe University, Theodor-Stern-Kai 7, D–60590 Frankfurt (Germany)
Tel. +49 69 6301 6526, Fax +49 69 6301 7108, E-Mail m.scholz@em.uni-frankfurt.de

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 47–53

Pathogenicity of Cardiopulmonary Bypass and Concepts of Leukocyte Filtration

Georg Matheis, Martin Scholz

Department of Thoracic and Cardiovascular Surgery, J.W. Goethe University, Frankfurt, Germany

In this contribution, definitions of the pathogenicity of cardiopulmonary bypass (CPB) in cardiac surgery will be provided. The pathogenicity of ischemia/reperfusion is discussed in detail by Krishnadasan et al. [1] in the next chapter. The clinically relevant impact of leukocyte filtration in the context of CPB will be covered subsequently. This short introduction therefore only outlines a number of leukocyte filtration concepts and their possible combinations in cardiac surgery.

Numerous studies on leukocyte filtration in cardiac surgery have focussed on various laboratory and clinical endpoints, and on a wide range of conditions, from routine coronary artery bypass surgery to heart transplantation. The evolution of the diverse leukocyte filtration protocols is based on our knowledge about leukocyte pathogenicity in cardiac surgery. Therefore, the rationale for the development of leukocyte filtration strategies must conceivably be derived from our knowledge about the specific pathomechanisms in CPB/reperfusion-associated tissue damage.

All patients undergoing general anesthesia are confronted with the consequences of this technique on leukocyte activation. Surgical trauma of varying degrees is a pathogenic factor that is known to activate leukocytes as well. CPB is a popular intervention that affects the whole body.

The inflammatory reaction of the whole body is a major contributor to leukocyte activation, and embolic events of various origin and quality are associated with CPB as well. In the context of CPB, aortic cross-clamping, i.e. cardiopulmonary ischemia, acts as an additional cardiopulmonary problem that is particularly important when reperfusion is initiated by releasing the aortic

cross clamp. Aortic cross-clamping can be avoided when CPB surgery is performed on a beating heart, i.e. emergency coronary artery bypass grafting (CABG) when additional iatrogenic global ischemia is to be avoided. Cardiotomy suction is a technique linked to CPB, and must be used with caution because direct retransfusion of large amounts of suction fluid consisting of blood, tissue debris (fat, bone marrow, cartilage, myocardium) and micro- and macrobubbles is a potent leukocyte activator, and, among many other problems, may result in lipid accumulation in the cerebral vasculature.

There are controversial reports on neutrophil activity and the impact of leukocyte filtration in this regard. For example, Thurlow et al. [2] postulate that filters are not capable of significantly depleting the neutrophil load generated during CPB, but may be capable of selectively removing the more activated forms. Own data reveal that the number of neutrophils with high levels of CD18 expression frequently seem to be reduced by leukocyte filters. However, phenotypic markers alone are not sufficient to draw conclusions on functional activity. Therefore, when addressing leukocyte-mediated pathology we should also assess functional aspects, such as neutrophil phagocytotic activity, oxidative burst and adhesion/transmigration to/through endothelial monolayers in vitro.

In the majority of cases a leukocyte filter simply has been placed in the arterial line instead of a standard arterial filter and employed throughout CPB. This seemingly rational approach may be unwise considering the decline in efficiency of leukocyte filters over time. On the other hand, leukocytes are particularly pathogenic during certain phases of the operation, such as reperfusion after release of the aortic cross clamp. This information has stimulated studies on leukocyte filtration in the arterial line at the time of maximum efficiency, i.e. when reperfusion injury takes place. Protocols to optimize onset and duration of so-called 'strategic leukocyte filtration' have evolved recently.

Studies have been performed in routine CABG to evaluate whether short-term leukocyte filtration initiated with or before onset of reperfusion after release of the aortic cross clamp may reduce reperfusion-associated myocardial damage. Further data compared the efficacy of three different filtration concepts to reduce CPB- and/or reperfusion-associated leukocyte pathogenicity.

It is well accepted that aberrant neutrophil activation during cardiac surgery with CPB is associated with postoperative complications [3–7] such as the systemic immune response syndrome (SIRS). Neutrophils are proposed to be activated by two major immunogenic mechanisms: (1) contact of circulating leukocytes with artificial surfaces, and (2) ischemia and reperfusion (oxidative stress, activation of endothelium) after release of the aortic cross clamp. A growing body of evidence exists for a beneficial role of leukocyte filters placed in the arterial line of the extracorporeal circuit [8].

Several authors have been able to show that reperfusion injury to cardiac and pulmonary tissue is diminished by leukocyte filtration in the arterial line [9–18] or the venous line [19], and that leukocyte filtration in the blood cardioplegia delivery system can afford improved myocardial protection. Despite these promising results, postoperative complications including SIRS, myocardial and pulmonary reperfusion injury, and cerebral damage, remain critical issues. Other pathomechanisms such as aberrant function of clotting factors and platelets, complement factors or proteinases, may not be directly affected by leukocyte filters. However, it should be considered that the potential of the leukocyte filtration procedure has never been systematically evaluated. Many groups used a leukocyte filter instead of a standard 40-μm filter in the arterial line throughout CPB without evidence of sufficient filter efficacy during the critical phase of heart and lung reperfusion.

In order to further evaluate the optimal timing of leukocyte filtration in the arterial line, we allocated patients of our clinic to four different groups in which the timing of arterial line filtration was modified. Briefly, CPB was done without or with leukocyte filtration initiated at onset of CPB, 10 min before aortic declamping, and starting with aortic declamping (see Matheis et al. [20] in this book). Another crucial issue in cardiac surgery is the leukocyte filtration of blood cardioplegia that may address a highly pathogenic situation with repetitive 20-min periods of ischemia followed by a short reperfusion phase during cardioplegic reinfusion. The coronary blood cannot be filtered by arterial line leukocyte filters during aortic cross-clamping unless the blood used for cardioplegia is drawn behind the arterial filter. While blood cardioplegia leukocyte filtration has been assessed by several groups [21–23], leukocyte filtration in the venous line is a novel approach, which has been reported to reduce neutrophil counts and IL-8 [19]. An overview of possible leukocyte filtration strategies is depicted schematically in figure 1. Whether a combination of arterial/venous line and blood cardioplegic leukocyte filtration has additional benefits remains unanswered, but seems likely because of the benefits shown by both isolated approaches.

The filtration of transfused blood products is required by law in several countries and is an adjunct to the concepts outlined above. The autologous blood collected in the cardiotomy reservoir at the end of CPB and chest drain blood can be retransfused, but they consist of numerous pathogenic components (fat, tissue debris) that may affect the outcome unless they are filtered. Current leukocyte filters frequently become occluded when used for chest drain or cardiotomy reservoir blood, and therefore limit this option. Despite the serious pathogenic effects of cardiotomy suction and chest drain fluid, the problem of leukocyte filtration in this highly contaminated blood remains unsolved.

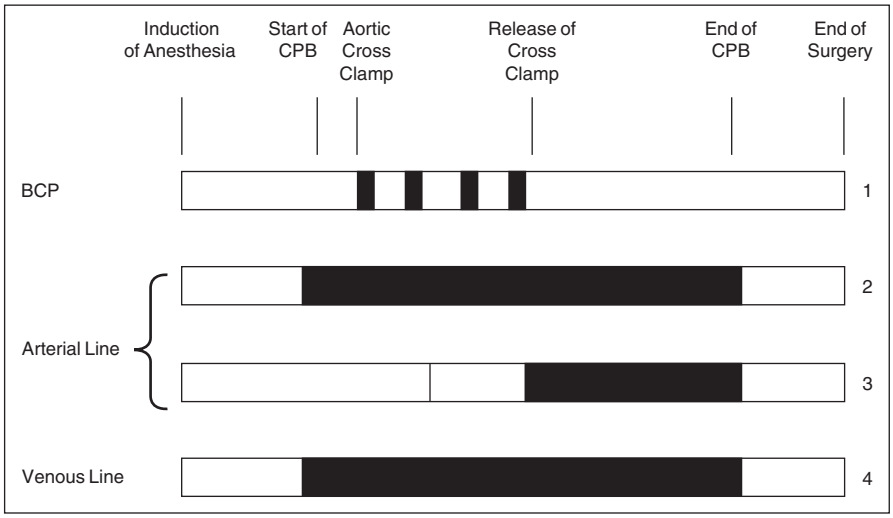


Fig. 1. Current leukocyte filtration concepts. 1 = Filtration of blood cardioplegia (BCP) solution, which is infused four times for about 20 min, as indicated by the black bars. 2, 3 = Filtration in the arterial line during the entire CPB period (2), started before the release of the cross clamp or immediately after its release (3). 4 = Filtration in the venous line during the entire CPB period.

If the efficiency of strategic leukocyte filters on release of the aortic cross clamp is not sufficient to remove a clinically relevant fraction of (activated) leukocytes, at least a change of leukocyte filters would be required prior to reperfusion. Despite legal reasons, particularly in the US, for using standard arterial line filters there is no conclusive evidence that these filters result in a clinical benefit when used in conjunction with membrane/hollow fiber oxygenators [24].

A recent study by Gu et al. [19] showed the feasibility of low flow filtration in the venous line. This technique may use the capacity of the existing arterial line leukocyte filters more effectively by perfusing this filter with a lower blood flow. The latter approach may become useful for evolving concepts of CPB without a reservoir. The question of the timing of leukocyte filtration remains open in this context and filtration in the venous line is largely unexplored.

Despite numerous modifications of CPB including heparin coating [25], protein coating [26], surface modification [27], pulsatile CPB [28], centrifugal pumps [29], myocardial damage due to reperfusion injury after release of the aortic cross clamp remains largely unaffected by these interventions, and has

been advanced mainly by the introduction of blood cardioplegic regimens [30]. The pathology of reperfusion injury appears to be largely based on oxidative stress and leukocyte-mediated disturbance of endothelial integrity. A considerable body of evidence exists regarding selective myocardial protection by leukocyte filters in the blood cardioplegia line [21–23]. If intermittent warm or cold blood cardioplegia is employed, several intervals of myocardial ischemia are followed by short (usually 2–3 min) periods of myocardial reperfusion during blood cardioplegia infusion. The use of leukocyte filters in this particular setting can be based on the same rationale as used for studying arterial line leukocyte filtration initiated with or shortly before the release of the aortic cross clamp.

Accordingly, an effective combination of interventions that may address different aspects of CPB/reperfusion-associated pathomechanisms seems to be a promising strategy to improve the clinical outcome after cardiac surgery with CPB. This assumption is underlined by data obtained in clinical studies with leukocyte filtration combined with heparin-coated CPB circuits versus heparin-coated circuits alone [31]. The results of the evaluation of optimal leukocyte filtration timing with noncoated circuits seem to confirm that heparin-coated circuits plus strategic leukocyte filtration during reperfusion is superior to leukocyte filtration alone, regardless of the timing strategy used.

In conclusion, the combination of interventions that address different pathomechanisms during CPB/reperfusion may further improve clinical outcome after cardiac surgery with the present technology.

References

1 Krishnadasan B, Griscavage-Ennis J, Aldea GS, Verrier ED: Reperfusion injury during cardiopulmonary bypass; in Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology. Basel, Karger, 2002, pp 54–77.

2 Thurlow PJ, Doolan L, Sharp R, Sullivan M, Smith B, Andersen LW: Laboratory studies of the effect of Pall extracorporeal leucocyte filters LG6 and AV6 on patients undergoing coronary bypass grafts. Perfusion 1996;11:29–37.

3 Hill GE: Cardiopulmonary bypass-induced inflammation: Is it important? J Cardiothorac Vasc Anesth 1998;12:21–25.

4 Rinder C, Fitch J: Amplification of the inflammatory response: Adhesion molecules associated with platelet/white cell responses. Cardiovasc Pharmacol 1996;27:6–12.

5 Cameron D: Initiation of white cell activation during cardiopulmonary bypass: Cytokines and receptors. J Cardiovasc Pharmacol 1996;27:1–5.

6 Kirklin JK, Westaby S, Blackstone EH, Kirklin JW, Chenoweth DE, Pacitico AD: Complement and the damaging effects of cardiopulmonary bypass. J Thorac Cardiovasc Surg 1983;86:845–857.

7 Butler J, Rocker GM, Westaby S: Inflammatory response to cardiopulmonary bypass. Ann Thorac Surg 1993;55:552–559.

8 Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W: Leukocyte depletion results in improved lung function and reduced inflammatory response after cardiac surgery. J Thorac Cardiovasc Surg 1996;112:494–500.

9 Breda MA, Drinkwater DC, Laks H, et al: Prevention of reperfusion injury in the neonatal heart with leukocyte-depleted blood. J Thorac Cardiovasc Surg 1989;97:654–665.
10 Bando K, Pillai R, Cameron DE, et al: Leukocyte depletion ameliorates free radical-mediated lung injury after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;99:873–877.
11 Wilson IC, Gardner TJ, DiNatale JM, Gillinow AM, Curtis WE, Cameron DE: Temporary leukocyte depletion reduces ventricular dysfunction during prolonged postischemic reperfusion. J Thorac Cardiovasc Surg 1993;106:805–810.
12 Allen SM, Pagano D, Bonser RS: Pall leukocyte depleting filter during cardiopulmonary bypass (letter). Ann Thorac Surg 1994;58:1560.
13 Coleman SM, Demastrie L: Leukocyte depletion reduces postoperative oxygen requirements (letter). Ann Thorac Surg 1994;58:1567–1568.
14 Johnson D, Thompson D, Mycyk T, Burbridge B, Mayers I: Depletion of neutrophils by filter during aortocoronary bypass surgery transiently improves postoperative cardiorespiratory status. Chest 1995;107:1253–1259.
15 Mihaljevic T, Tonz M, von Segesser LK, et al: The influence of leukocyte filtration during cardiopulmonary bypass on postoperative lung function: A clinical study. J Thorac Cardiovasc Surg 1995;109:1138–1145.
16 Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W: Clinical performance of a high efficacy rapid flow leukocyte removal filter for leukocyte depletion of heparinized cardiopulmonary bypass perfusate. Perfusion 1995;10:425–430.
17 Gu YJ, Obster R, Haan J, Gallandat Huet RC, Eijgelaar A, van Oeveren W: Biocompatibility of leukocyte removal filters during leukocyte filtration of cardiopulmonary bypass perfusate. Artif Organs 1993;17:660–665.
18 Komai H, Naito Y, Fujiwara K, Takagaki Y, Noguchi Y, Nishimura Y: The protective effect of a leucocyte removal filter on the lung in open-heart surgery for ventricular septal defect. Perfusion 1998;13:27–34.
19 Gu YJ, de Vries AJ, Vos P, Boonstra PW, van Oeveren W: Leukocyte depletion during cardiac operation: A new approach through the venous bypass circuit. Ann Thorac Surg 1999;67:604–609.
20 Matheis G, Simon A, Scholz M: Strategic leukocyte filtration: Clinical and experimental experience; in Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology. Basel, Karger, 2002, pp 101–110.
21 Suzuki I, Ogoshi N, Chiba M, Komatsu T, Moizumi Y: Clinical evaluation of a leucocyte-depleting blood cardioplegia filter (BC1B) for elective open-heart surgery. Perfusion 1998;13:205–210.
22 Kofsky ER, Julia PL, Buckberg GD, Quillen JE, Acar C: Studies of controlled reperfusion after ischemia. XXII. Reperfusate composition: Effects of leukocyte depletion of blood and blood cardioplegic reperfusates after acute coronary occlusion. J Thorac Cardiovasc Surg 1991;101: 350–359.
23 Schmidt FE Jr, MacDonald MJ, Murphy CO, Brown WM 3rd, Gott JP, Guyton RA: Leukocyte depletion of blood cardioplegia attenuates reperfusion injury. Ann Thorac Surg 1996;62: 1691–1696.
24 Matheis G: Arterielle Filter für den Extrakorporalen Kreislauf; in Extrakorporale Zirkulation – wissenschaftlich begründet? Lübeck Deutsche Gesellschaft für Thorax-, Herz- und Gefässchirurgie, 2001.
25 Gott JP, Cooper WA, Schmidt FE Jr, Brown WM 3rd, Wright CE, Merlino JD, Fortenberry JD, Clark WS, Guyton RA: Modifying risk for extracorporeal circulation: Trial of four antiinflammatory strategies. Ann Thorac Surg 1998;66:747–753.
26 Wimmer-Greinecker G, Matheis G, Martens S, Oremek G, Abdel-Rahman U, Moritz A: Synthetic protein treated versus heparin coated cardiopulmonary bypass surfaces: Similar clinical results and minor biochemical differences. Eur J Cardiothorac Surg 1999;16:211–217.
27 Gu YJ, Boonstra PW, Rijnsburger AA, Haan J, van Oeveren W: Cardiopulmonary bypass circuit treated with surface-modifying additives: A clinical evaluation of blood compatibility. Ann Thorac Surg 1998;65:1342–1347.
28 Taylor KM: Pulsatile reperfusion: The effects on coronary blood flow and myocardial ischemic injury. J Thorac Cardiovasc Surg 1983;85: 941–942.

29 Parolari A, Alamanni F, Naliato M, Spirito R, Franze V, Pompilio G, Agrifoglio M, Biglioli P: Adult cardiac surgery outcomes: Role of the pump type. Eur J Cardiothorac Surg 2000;18:575–582.
30 Buckberg GD: Oxygenated cardioplegia: Blood is a many splendored thing. Ann Thorac Surg 1990;50:175–177.
31 Matheis G, Scholz M, Gerber J, Abdel-Rahman U, Wimmer-Greinecker G, Moritz A: Leukocyte filtration in the early reperfusion phase on cardiopulmonary bypass reduces myocardial injury. Perfusion 2001;16:43–49.

Dr. med. G. Matheis, Jostra Medizintechnik AG, D–72145 Hirrlingen (Germany)
Tel. +49 7478 921 418, Fax +49 7478 921 400, E-Mail georg.matheis@jostra.net

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 54–77

Reperfusion Injury during Cardiopulmonary Bypass

Baiya Krishnadasan, Jeanette Griscavage-Ennis, Gabriel S. Aldea, Edward D. Verrier

Department of Surgery, Division of Cardiothoracic Surgery,
University of Washington, Seattle, Wash., USA

Ischemia-reperfusion (I/R) injury is a manifestation of the acute inflammatory response initiated by overlapping cascades of inflammatory mediators expressed at both local and systemic levels. It results in increased capillary permeability, accumulation of interstitial fluid, leukocytosis, disturbances in coagulation, and ultimately, organ dysfunction. Commonly affected organs include the lung, gastrointestinal tract, kidney and brain, with ensuing acute lung injury requiring mechanical ventilation, decreased hepatic synthetic capacity, acute renal insufficiency and neuropsychiatric dysfunction. I/R injury can be regional, such as following percutaneous transluminal coronary angioplasty or thrombolytics for acute myocardial infarction, or it can be global, following cardiopulmonary bypass (CPB) with ischemic cardiac arrest. A common phenomenon in cardiac surgery, I/R injury leads to a spectrum of derangements from arrhythmias and postischemic myocardial dysfunction to severe irreversible abnormalities like infarction. Despite improvements in technology, open-heart surgery is associated with a requisite ischemic period related to CPB and application of the aortic cross clamp. Reperfusion results upon revascularization and removal of the aortic cross clamp. The damaging effects of I/R begin during the period of reperfusion and may only become manifest hours after CPB. A post-pump syndrome was first identified by Kirklin [1] involving both humoral and cellular systemic responses. This syndrome exists as a continuum from mild to severe injury correlated with the duration of the surgical ischemic period.

Although some components of I/R injury are leukocyte-independent, the underlying molecular events are largely mediated through neutrophil-endothelial

interactions. Because of the importance of leukocytes in I/R injury, attempts have been made to clinically lessen injury by depleting leukocytes. Leukocytes can be removed by either physical or pharmacologic mechanisms. Although some of these depletion strategies are successful, others are not, and the role of leukocyte depletion in cardiovascular surgery is still controversial. Despite the controversy, it is apparent that depletion of leukocytes can only partially prevent reperfusion injury. Thus, no discussion of I/R injury would be complete without considering leukocyte-independent injury. This chapter will review both leukocyte-independent and leukocyte-dependent reperfusion injury at a molecular level and will critically evaluate the successes and failures of leukocyte depletion in the literature.

Mechanisms of Leukocyte-Independent Ischemia-Reperfusion Injury

Leukocyte-independent I/R involves reactive oxygen species (ROS), release of soluble arachidonic acid metabolites, endothelial peptides, activation of the humoral amplification systems, and cytokines. At a molecular level, CPB results in measurable increases in all of these mediators. In addition, the endothelium becomes substantially altered expressing a phenotype that initiates and amplifies inflammation and coagulation. Many of these factors have overlapping activities and serve to activate or potentiate the effects of leukocytes. Therefore, their functions cannot be considered mutually exclusive. This discussion will consider the molecular mechanisms for each component separately, but will indicate potential interactions where relevant.

Reactive Oxygen Species

Molecular oxygen is essential for maintaining cellular viability. Paradoxically, it is also a primary contributor to tissue damage following reperfusion. Evidence for this incongruity comes from animal experiments in which reperfusion of an anoxic heart with oxygenated solution increased myocardial injury compared to reperfusion with a hypoxic solution [2]. While cultured cells and tissues may survive hypoxic insult for many hours, severe, prolonged hypoxia will eventually result in cell death. Throughout the hypoxic period, physiologic changes occur in the cell leading to alterations in function and increasing susceptibility to further injury. This phenomenon is termed ‘hypoxic priming’ and is mediated through second messengers like intracellular calcium, phosphatidic acids, cyclic adenine monophosphate (cAMP) and ROS. In addition, specific cells, including neutrophils, macrophages and endothelial cells, are primed to participate as effectors in the inflammatory response.

During the hypoxic period the cell's energy stores are depleted (fig. 1). There is a drop in the transmembrane potential and the cell is no longer able to maintain proper ion gradients across its membranes. This precipitates increased intracellular sodium concentrations and cellular swelling. Intracellular calcium also increases due to increased permeability of calcium channels and decreased ATP-dependent export of calcium from cells. The resulting calcium overload stimulates contraction of the intracellular skeleton and alterations in cell shape. Increased calcium also activates phospholipases to release free fatty acids from cell and organelle membranes, leading to the activation of the arachidonic acid cascade. Concomitantly, the depletion of cellular ATP results in an elevated concentration of AMP. AMP is catabolized to adenosine, inosine and finally hypo xanthine [3]. Another consequence of hypoxia is the depletion of endogenous antioxidant defenses such as glutathione (GSH). GSH maintains cellular proteins in their reduced form; however, this function results in its oxidation to GSSG. In order for a cell to maintain the appropriate reducing environment, a constant recycling of GSSG to GSH must occur, a process that requires NADPH [4]. NADPH is depleted by hypoxia. Thus, the biological changes associated with sublethal hypoxia results in cells that are 'primed' to respond to reintroduction of oxygen.

Reperfusion is defined as the restoration of oxygen resulting in the generation of free radical oxygen species or ROS. ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical (•OH). There are numerous sources of intracellular ROS, including endoplasmic reticulum, plasma and nuclear membranes, mitochondrial electron transport systems and soluble enzymes. The major source of superoxide in postischemic tissues appears to be the enzyme xanthine oxidase [5]. Extracellular sources include autooxidation of catecholamines and the activated neutrophil respiratory burst. ROS generated in limited amounts under normal aerobic conditions are rapidly neutralized by intracellular enzymatic free radical scavengers, which act as the natural antioxidant defense. The main components of this defense include cytochrome oxidase, superoxide dismutase, catalase, GSH, vitamin E and vitamin C. However, during hypoxic 'priming' there is a buildup of hypoxanthine and xanthine, two substrates for the enzyme xanthine oxidase. Upon reperfusion, the remaining substrate for xanthine oxidase, molecular oxygen is supplied, resulting in a burst of superoxide and hydrogen peroxide production. The resultant imbalance between ROS production and the availability of natural antioxidant scavengers during postischemic reperfusion sets the stage for tissue injury. The magnitude of the oxygen free radical burst is dependent on levels of xanthine, which increase with the length and severity of the preceding cellular ischemia.

Following reoxygenation, ROS generated within cells may directly damage cell and organelle membranes, denature proteins and disrupt chromosomes [6].

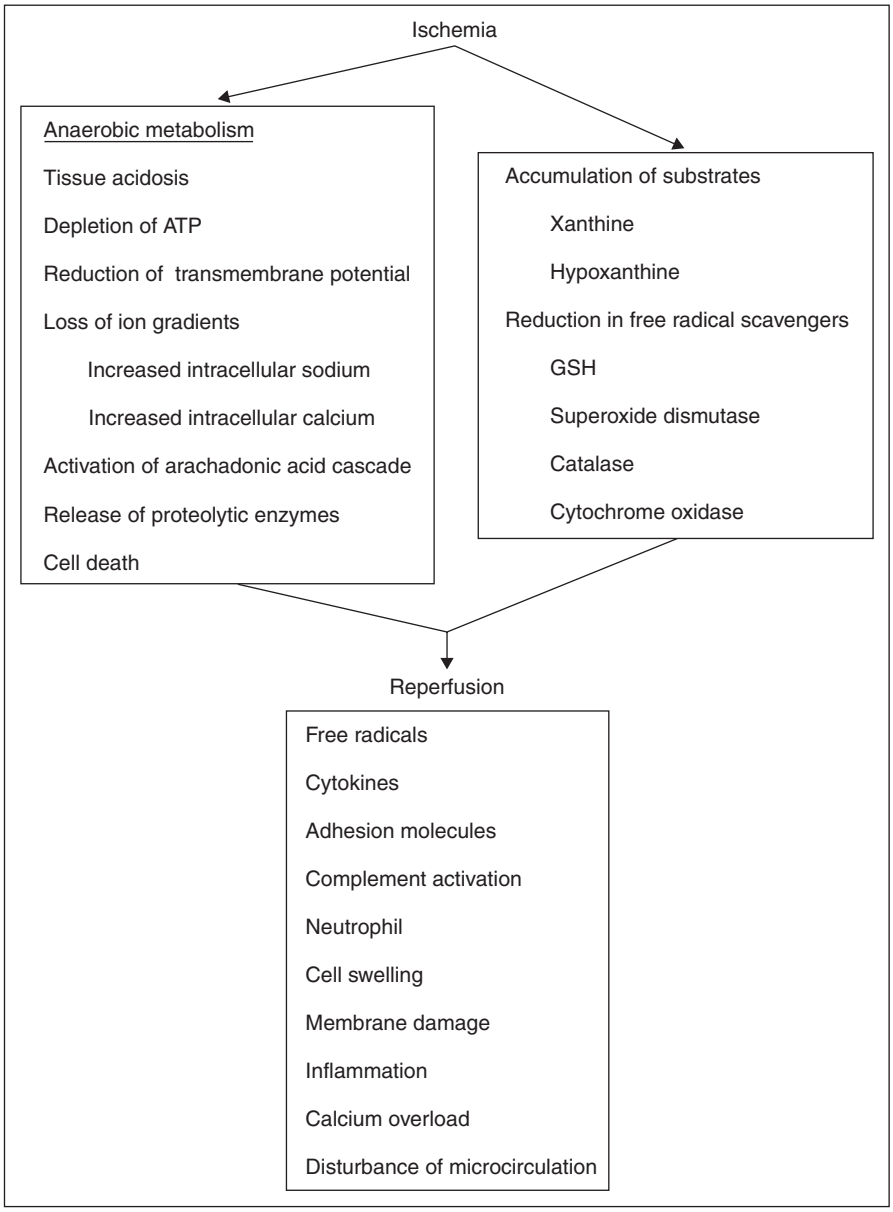


Fig. 1. Molecular events during ischemia and reperfusion. During ischemia, cellular energy stores are depleted, resulting in the 'priming' of cells to respond to the reintroduction of oxygen. Reperfusion triggers a free radical burst which, combined with depleted anti-oxidant defenses, leads to I/R injury.

They may escape from cells and injure adjacent cells as well as enter the circulation to produce a systemic effect. While this damage may occur in all cells, endothelial cells in the microvascular circulation are especially affected. ROS may also act indirectly as second messengers to stimulate the acute inflamm tory response. ROS are potent stimulators of protein synthesis by activating transcription factors, such as nuclear factor-κB (NF-κB). Activation of NF-κB leads to enhanced production of the proinflammatory cytokines, TNF-α, IL-1 and IL-8, and surface adhesion molecules. A more detailed discussion of each of these mediators, and how they contribute to I/R injury will follow.

There are numerous studies demonstrating the involvement of ROS in I/R injury [7]. Electron paramagnetic resonance spectroscopy data demonstrate the presence of ROS within the first 5 min of heart reperfusion [8–11]. Several studies document the benefit of pretreatment with xanthine oxidase inhibitors or oxygen free radical scavengers before and during the ischemic period. In rodent heart [12], lung, and heart-lung transplant [13] models, SOD provided improved recovery of tissue following the I/R insult. I/R injury was also significantly reduced following depletion of ROS by catalase, mercaptopropionylglycine or dimethylthiourea or preventing formation with desferrioxamine, allopurinol or oxypurinol. Although activated neutrophils generate significant quantities of ROS, an additional protective effect was demonstrated with a liposomal preparation of SOD used in concert with leukocyte depletion, providing support for the notion that leukocytes are not the only source of ROS [14].

Release of Arachidonic Acid Metabolites and Platelet-Activating Factor

Arachidonic acid metabolites either can directly or indirectly injure cells via stimulation of cAMP synthesis. Arachidonic acid is a precursor molecule that is transformed into potent mediators with far-ranging effects. Eicosanoids, a group of biologically active compounds derived from arachidonic acid via the actions of the enzyme cyclooxygenase, are detected in increased concentrations in association with CPB. Two important eicosanoids, prostacyclin and thromboxane, possess opposing effects. Thromboxane A_2 (TxA_2) is a platelet-aggregating and vasoconstrictive substance. Thromboxane B_2, a stable metabolite of TxA_2 can be detected in increasing amounts during CPB [15, 16]. Blockade of thromboxane receptors or inhibition of thromboxane synthesis has consistently been shown to inhibit pulmonary injury and to improve ventricular function after CPB [17]. In contrast, prostacyclin release results in vasodilation and a quiescent endothelium. The final group of arachidonic acid mediators with pronounced proinflammatory effects are the leukotrienes. Leukotrienes are among the most potent chemoattractants for neutrophils inducing augmented adherence, degranulation and oxygen radical formation in these cells. ROS

stimulate the initiation of the arachidonic acid cascade leading to the synthesis of TxA_2 by endothelial cells and leukotrienes by neutrophils. Products of the complement cascade also stimulate the arachidonic acid pathway.

Another important inflammatory mediator produced by endothelial cells is platelet activating factor (PAF). PAF is a glycerol phospholipid formed from endothelial cell, platelet and granulocyte plasmalemmal phosphatidylcholine. Under normal physiological conditions, PAF is minimally expressed; however, I/R results in PAF release from neutrophils and monocytes and increased expression by endothelial cells [18]. As its name suggests, PAF is a potent activator of platelets. In addition, PAF mediates other diverse biological activities, including neutrophil activation and chemotaxis, alterations in vascular permeability and negative ionotropy. Numerous studies support a significant role for PAF in I/R injury. Not only do PAF levels increase 350% during CPB [19], but PAF antagonists have been shown to be cardioprotective and improve survival when administered before onset of reperfusion [20–22]. Global dysfunction is due to the effect of inflammatory mediators such as PAF, thromboxanes, leukotrienes, and endothelins that are released during reperfusion. Antagonizing a central inflammatory mediator such as PAF, as adjunct treatment with currently used reperfusion therapies, improves cardiovascular function and survival in animals [22] and may provide similar protective effects in human trials.

Endothelial Peptides

Endothelial cells cover the luminal surface of capillaries and synthesize vasodilators and vasoconstrictors the balance of which helps to maintain the patency of blood vessels. Prostacyclin and nitric oxide cause vasodilation through the relaxation of underlying vascular smooth muscle. Another important vasorelaxant produced by the endothelium is adenosine. The endothelial arsenal of vasoconstrictor substances includes endothelins, TxA_2 and angiotensin II. One of the many effects of hypoxia and reperfusion on endothelial cells is increased vasoconstriction. I/R results in the downregulation or inactivation of prostacyclin, adenosine and nitric oxide as well as unopposed vasoconstriction that worsens the ischemic insult. Injured or ischemic endothelial cells generate endothelin-1, an extremely potent vasoconstrictor. Endothelin-1 is a 21-amino-acid peptide that is released by endothelial cells, vascular smooth muscle cells and cardiomyocytes. Growth factors, cytokines and various vasoactive substances including thrombin, TGF-β, oxidized low-density lipoprotein, angiotensin II and bradykinin stimulate its expression. Hypoxia-reoxygenation stimulates a greater than 198% increase in endothelin production [23]. Increased levels have also been demonstrated following CPB. A role for endothelin in coronary vasoconstriction and myocardial ischemia is suggested

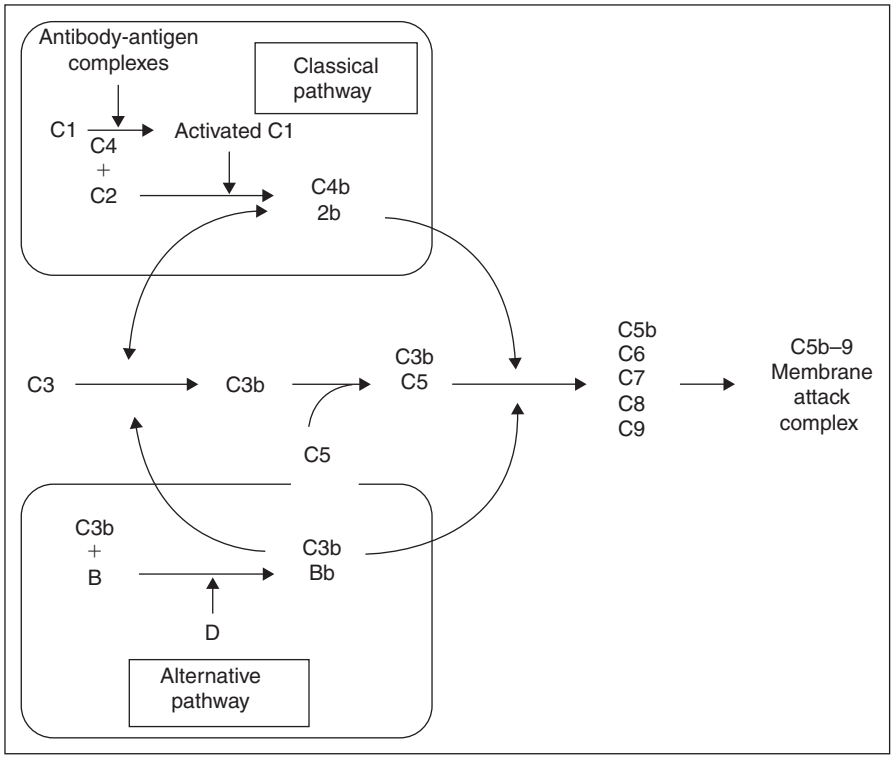


Fig. 2. Complement cascade. The two pathways of complement activation are triggered independently during CPB and I/R. The pathways converge at the level of C3 and culminate in the formation of the membrane attack complex (C5b–9).

by studies showing a reduction in infarct size and improved recovery of myocardial performance with agents that block endothelin activity (monoclonal antibody, endothelin-converting enzyme inhibitor, endothelin receptor antagonist) [24].

Humoral Amplification Systems

The humoral amplification system consists of the activation of the complement cascade (fig. 2), the coagulation pathway, kallikrein-bradykinin and fibrinolytic systems. Complement activation is a central component of the whole-body inflammatory response associated with CPB and the humoral amplification system. Several lines of investigation support a role for complement in the pathogenesis of I/R injury. Following CPB, complement components are localized in the infarcted myocardium and their depletion by cobra

venom factor or their inhibition by C1 esterase or soluble complement receptor attenuates I/R injury.

The most prominent early response to CPB and I/R is massive complement activation [25]. There are two pathways of activation of the complement cascade, the classical and the alternative pathway. While both pathways are triggered independently, they converge into a common pathway at the level of C3, generating the anaphylatoxins, C3a and C5a. Ultimately activation of complement leads to the formation of the membrane attack complex (C5b–9). The classical pathway requires the formation of an antigen-antibody complex and in cardiovascular surgery is activated by heparin-protamine complexes, blood/air interfaces and endotoxin. The alternative pathway is activated by contact activation with the nonphysiologic surfaces of the bypass circuit as well as by activated pericardium and cardiotomy suction blood. Another source for the activation of complement is the interaction between C1 and the damaged mitochondrial membranes. In the context of reperfusion injury, in vitro studies have shown that hydrogen peroxide directly increases C3 and C5 levels. Increased levels of C5a, C3a and C5b–9, but not C4b/c are observed during CPB [26]. Lack of C4b/c suggests that the classical pathway is not involved and that activation of complement during CPB involves the alternate pathway. A second phase of complement activation occurs 2–3 days after surgery and is associated with activation of the classical pathway.

Once activated, complement stimulates a variety of responses contributing to the inflammatory response. C3a, C4a and C5a all increase vascular permeability by acting directly on smooth muscle cells and by eliciting histamine release from mast cells and basophils. These mediators also contribute to eicosanoid production and the activation of platelets. C5a can cause the conversion of xanthine dehydrogenase to xanthine oxidase in hypoxic endothelial cells [27] priming cells for an oxidative burst upon reperfusion. The terminal C5b–9 membrane attack complex can directly induce myocardial injury [28], and attenuate endothelium-derived relaxation of vascular smooth muscle cells [29–31]. In addition, the anaphylatoxins mediate leukocyte chemotaxis and promote leukocyte adhesion and activation. Neutrophils in turn further stimulate the complement cascade potentiating the inflammatory cycle.

Inhibition of complement activation at C5a would prevent the formation of subsequent proinflammatory molecules while still allowing for the generation of upstream molecules such as C3b, a critical mediator of bacterial opsonization as well as immune complex solubilization and clearance. For this reason, many preclinical and clinical studies have been designed utilizing monoclonal antibodies directed against C5a [32, 33]. One such trial used a recombinant, humanized antibody directed against C5a. This complement inhibitor resulted in a 40% reduction of myocardial injury, a 1-unit reduction in postoperative

blood loss and an 80% reduction in new cognitive deficits when used in patients undergoing CPB [34]. Another approach to inhibiting complement activation involves the use of heparin-coated CPB equipment, with variable results.

Thrombin is a small peptide involved in the humoral amplification system. During CPB, levels of thrombin are increased. Traditionally, the role of thrombin has been associated with the conversion of fibrinogen to fibrin resulting in clot formation. More recently it has been shown that complement triggers the release of thrombin, resulting in amplification of inflammation. Through interactions with its receptor, thrombin induces various cell-stimulating events, notably on endothelial cells, with important proinflammatory consequences. Thrombin induces prostacyclin, PAF synthesis and P-selectin expression by human umbilical vein endothelial cells (HUVECs) [35]. These events are rapid and do not require de novo protein synthesis. Studies have also shown that thrombin is an important regulator of polymorphonuclear leukocyte adhesion to endothelial cells. Neutrophilic infiltration is due to interactions of blood cells with cytokines and adhesion molecules synthesized and expressed by endothelial cells. The production of both cytokines (IL-8) and adhesion molecules (E-selectin) requires protein synthesis [36, 37]. In addition to its role in acute inflammation, thrombin may be an important stimulus in chronic inflammation. Thrombin is known to be chemotactic for monocytes and supports monocyte adhesion by inducing the expression of the adhesion molecules, ICAM-1 and VCAM-1.

Although it is not directly related to reperfusion injury, the negatively charged surfaces of the CPB machine lead to the generation of kinins. Hageman factor (factor XII) is a central mediator that is activated by foreign surfaces. It results in coagulation through the activation of factors XI–XIa, as well as the production of kinins. The kinins are a discrete group of peptides that increase vascular permeability and induce vasodilation. The clinical consequences of kinin generation are hypotension, shock and end organ damage. Kallikrein is also produced by activated factor XII amplifying the production of kinins. Clinically, it is possible to block kallikrein with the serine protease inhibitor, aprotinin.

Cytokines

Cytokines are hormone-like glycoproteins that function to communicate between inflammatory cells. They play a central role in the pathogenesis of shock and of multiple organ failure during sepsis. Among their many actions, cytokines initiate and amplify the inflammatory response. This includes the induction of leukocyte proliferation, differentiation, maturation and function, as well as directing cytotoxic effects and the stimulation of additional ROS. CPB is associated with cytokine release, which is thought to be due to a wide range

of factors such as I/R, complement activation, and endotoxemia. The most commonly studied cytokines in relationship to CPB are tumor necrosis factor-α (TNF-α), and the interleukins, IL-1β, IL-6, IL-8 and IL-10. In the context of I/R, there are multiple cellular sources for these cytokines, including endothelial cells, neutrophils and monocytes.

There is massive redundancy in the actions of these cytokines. During CPB, TNF-α levels are increased twofold by 1 h and return to baseline by 24 h. In vitro, C5a, Il-1β, gram-negative endotoxin and gram-positive bacterial products are potent stimulators of TNF-α release. TNF-α contributes to the induction of the acute-phase response with increased synthesis of hepatic proteins and glycoproteins [38]. Il-1β production can be triggered by complement anaphylatoxins, endotoxin and monocyte adherence to foreign surfaces. Il-1β appears to function in a paracrine manner to induce the synthesis of IL-6 by monocytes. Both IL-6 and IL-8 are released from endothelial cells under hypoxic conditions. IL-6 levels peak at 4 h post-bypass and remain elevated at 24 h [39]. IL-6 stimulates acute-phase response proteins, whereas IL-8 is a potent neutrophil chemoattractant. Therefore, a discussion of the role of IL-8 in I/R injury is best considered with neutrophil-mediated injury. IL-10 is unique in that it is considered an anti-inflammatory cytokine.

Although not a cytokine, bacterial endotoxin is a potent inducer of many cytokines, especially TNF-α, and is a powerful activator of the inflammatory response. Endotoxin is a lipopolysaccharide derived from the walls of gram-negative bacteria. In addition to eliciting cytokines, it functions to activate complement (both classical and alternative pathways), stimulate the oxidative burst of neutrophils and increase adhesiveness of vascular endothelial cells. There are many possible sources of endotoxin during CPB, but the gut is most often implicated [17]. In CPB, splanchnic vasoconstriction leads to intestinal mucosal injury and subsequent leakage of endotoxin into the blood stream. High systemic concentrations of endotoxin are observed post-CPB [40, 41]. Plasma endotoxin is maximal at 90 min, beginning to decline by 6 h and gradually falling through the 7th postoperative day [42]. The presence of endotoxin is important because it may explain (in part) the activation of the complement cascade via the alternative pathway and the increase in cytokine levels.

Attempts to modulate reperfusion injury by blocking arachidonic acid metabolites, complement, thrombin, cytokines, and chemokines have been limited by the tremendous redundancy of these systems. The effects of all of these cascades are overlapping; therefore blocking one of them does not necessarily result in a marked decrease in injury. Although these humoral factors may not be useful therapeutic targets, an understanding of the interactions between these peptides and the inflammatory milieu during CPB will improve future efforts to attenuate reperfusion injury.

Mechanisms of Cell-Mediated Reperfusion Injury

As is clear from the preceding discussion, reperfusion injury is partially mediated through humoral mechanisms. These soluble mediators act independently and in concert with cellular processes to produce injury. Blocking the humoral response does not entirely abrogate reperfusion injury. Similarly, blocking the cell-mediated response does not totally block injury.

The redundancy and close interaction between these two systems make both in vitro and in vivo study of I/R injury challenging. Efforts to study reperfusion injury in vivo primarily focus on the consequences of injury at the organ level. These studies typically report gross changes in histology, weight, messenger RNA or protein levels. Although these changes provide insight into the repercussions of I/R injury, they do not examine the causative molecular mechanisms. Elucidation of molecular mechanisms mediating I/R injury requires in vitro studies in isolated cell cultures. An important limitation of in vitro studies is the inability to reconstitute a physiologic environment and therefore to explain the complex consequences of reperfusion injury seen in vivo.

This section will discuss both in vitro and in vivo work investigating cell-mediated reperfusion injury. Fundamental to this discussion is an understanding that leukocytes do not act independently and that their actions are directed by the physiologic environment. A basic understanding of neutrophil biology is essential to an understanding of cell mediated-reperfusion injury. The interaction between neutrophils and endothelial cells will also be discussed in detail. The mediators that attract and activate neutrophils, some of which have been discussed in the previous section, will be examined. These discussions will focus on mediators and interactions that have been targeted by physicians in clinical practice. Therefore, some subjects will be given more emphasis than others, reflecting the interests of clinicians and not necessarily the importance of particular mediators.

Neutrophil Biology

Neutrophil is a specific term describing a type of peripheral leukocyte that is highly specialized for one function: the phagocytosis and destruction of foreign antigens. Two other terms are commonly used to describe neutrophils, granulocyte and polymorphonuclear leukocyte. These terms are general descriptors for a class of peripheral leukocytes, which includes eosinophils and basophils, and are not specific for the type of cell involved in reperfusion injury. The correct term describing the cell of interest in I/R injury is neutrophil.

Neutrophils differentiate from pluripotent stem cells located in the bone marrow. The earliest recognizable precursor of the mature neutrophil is the

Table 1. Bone marrow leukocyte content (percentage of neutrophil precursors in adult bone marrow)

Cell type	Mean, %
Myeloblast	2
Promyelocyte	5
Myelocyte	12
Metamyelocyte	22
Mature PMNs	20
Lymphocytes	10
Other polymorph precursors	19

myeloblast. The myeloblast is a small cell with a large nucleus, several nucleoli and conspicuous absence of granules in the cytoplasm. It is difficult to distinguish between myeloblast precursor cells under a light microscope although electron microscopy can distinguish subtle changes in the cytoplasm that signal that this stem cell will eventually differentiate into a neutrophil. The promyelocyte is the earliest cell that can be identified with certainty as a neutrophil precursor. These cells are distinguished by the deeply basophilic nucleus, which contains the beginnings of azurophilic granules. The myelocyte and metamyelocyte represent more mature forms in neutrophil development and consequently they contain the full complement of cytoplasmic proteins. The difference between these late precursors and the mature neutrophil is related to subtle differences in nuclear organization.

The adult human bone marrow contains all of the precursors of the neutrophil lineage (table 1). Progression of cells from the myeloblast to the neutrophil takes 7–10 days in the unstimulated state; however, in the stimulated host, i.e. infection or trauma, the progression from myeloblast to mature neutrophil may be much quicker. The maturation of these cells is controlled by growth factors, such as granulocyte macrophage colony-stimulating factor (GM-CSF), which have become useful clinical tools particularly in patients undergoing high-dose chemotherapy. Nearly 50% of normal adult bone marrow cells represent mature or nearly mature neutrophils. These cells can quickly be recruited and represent 30 times the number of neutrophils that are normally present in the circulating blood. The signals that cause the recruitment of this potent reserve of cells are now being elucidated and some of these peptides will be discussed in the following sections.

The mobilization of the enormous bone marrow stores of neutrophils has direct implication for clinical attempts at leukocyte depletion. Several clinical studies have shown no benefit to using leukocyte filtration. These studies used leukocyte filters during CPB and used only one filter in series with the circuit

with no decrease or very little decrease in neutrophil counts [39, 43, 44]. Other groups have been able to deplete neutrophil counts successfully using several filters in parallel or in series [45, 46]. The success of leukocyte filters is dependent on adequate neutrophil depletion. Using only one filter in the circuit may not be sufficient to achieve neutrophil depletion. A second issue that must be considered in using leukocyte filters is the appropriate timing of filtration. During CPB, there is a reproducible decrease in neutrophil counts. This nadir in neutrophil counts is secondary to inflammatory activation and demargination of neutrophils caused by the foreign surfaces of the bypass circuit. The bypass circuit causes the release of soluble mediators, discussed in detail in the first section, that activate neutrophils. These same mediators also result in the release of the bone marrow store of neutrophils. A peak in neutrophil counts occurs early after bypass, reflecting the combined effects of neutrophil demargination and release from the bone marrow. The ideal time to employ leukocyte filtration would be both during and immediately after CPB, when neutrophil counts and activity are maximal.

The existence and function of the neutrophil have only been appreciated for the last century. At the turn of the century a Russian zoologist, Elie Metchnikoff, hypothesized that the phagocytic activity of unicellular organisms was also likely to be the main host defense mechanism in multicellular organisms [47]. This hypothesis was expanded and confirmed in the following 20 years primarily with the use of microscopic analysis. The neutrophil was identified as the primary effector cell in host defense against microorganisms and subsequently various disease states were identified associated with neutrophil dysfunction or absence. In the past two decades there has been a renewed interest in neutrophil biology that has focused on the role of neutrophils in noninfectious inflammatory states, such as the acute respiratory distress syndrome. The investigation of these inflammatory processes has led to a better understanding of neutrophil chemotaxis, activation and function.

There are approximately 5×10^9 neutrophils in one liter of human blood, making them by far the most common leukocyte in the human circulatory system. Mature neutrophils are uniform cells measuring 12–15 μm under the microscope. These cells are highly differentiated and have unique structural features. The nucleus is perhaps the most characteristic feature of the neutrophil; it is multilobed and stains purple with Wright's stain. Nuclear material is clumped, suggesting that these cells are differentiated and do not divide. This does not mean that the genome is silent and, in fact, the neutrophil is metabolically active despite its differentiation.

Electron-microscopic examination of neutrophil cytoplasm reveals the presence of mitochondria, endoplasmic reticulum and granules. Periodic acid-Schiff staining of the cytoplasm reveals the presence of large quantities of

Table 2. Enzymes found in neutrophil granules

DNAase II
Acid ribonuclease
Lipase
Phospholipase A and B
Acid Phosphatase
α-Amylase
α- and β-Glucosidase
β-Glucuronidase
Elastase
Neutrophil protease
Collagenase
Cathepsin D
Myeloperoxidase
Lactoferrin

glycogen, indicating that phagocytosis occurs via a glycolytic pathway that is dependent on an extracellular source of glucose. The most prominent and distinctive cytoplasmic constituents, however, are the granules. Two types of granules have been described, azurophilic and specific granules.

Azurophilic granules represent 10–20% of the total population of granules, and as their name suggests, they stain purple with Romanovsky dye. Electron microscopy reveals that they are dense, measuring approximately 0.5 μm and surrounded by a unit membrane. The constituents of the azurophilic granules include various hydrolytic enzymes that are active at pH 5 (table 2). These enzymes break down the cellular products of dead cells into their basic building blocks including amino acids, sugars and individual nucleotides. Two of the enzymes in the granule, lysozyme and myeloperoxidase, are capable of killing cells. Lysozyme can digest the mucopeptide coat of bacteria and myeloperoxidase converts its substrate, hydrogen peroxide, into powerful oxidants. The remaining enzymes are not generally lethal to cells although they may cause significant damage to the extracellular matrix.

Specific granules measure 0.2 μm, stain faintly pink, are relatively electron lucent and are surrounded by a unit membrane. Specific granules contain lysozyme and lactoferrin. Lactoferrin is a protein originally found in milk with a heme prosthetic moiety. It has extraordinary affinity for ferric iron, a required product for bacterial growth. In addition it is thought that lactoferrin may directly kill bacteria.

Clinical studies have correlated levels of various neutrophil granule products with leukoctye activation during CPB. Investigators have reported increased elastase [48, 49], lactoferrin [50] and myeloperoxidase [51]. Both

elastase and lactoferrin are relatively ubiquitous enzymes and are not exclusively found in azurophilic or specific granules. Therefore, it is difficult to convincingly correlate increased levels of these enzymes with specific neutrophil activation, particularly in the proinflammatory environment produced by CPB. Nevertheless, these granule products have become accepted markers for neutrophil activation in human studies. Elastase levels may be more reliable as they seem to fluctuate more reproducibly with CPB.

Myeloperoxidase is an excellent marker for neutrophil activation. If myeloperoxidase activity is measured in tissue before and after the inflammatory insult, the results provide a reliable measure of neutrophil infiltration and activity. Blood levels of myeloperoxidase are an insensitive measure of neutrophil activation because neutrophils are typically not activated in the serum. In addition, the products of their granules would be diluted in the blood. Injury may result in elevated myeloperoxidase activity in the plasma, but this activity represents only a small fraction of the total seen in tissue. Clinically this presents a problem because it is difficult to justify taking multiple tissue biopsies to measure levels of myeloperoxidase.

Other neutrophil cell products have also been used to monitor inflammation [52]. These enzyme levels may reveal gross changes in neutrophil activation in the blood, but because they are nonspecific serum markers, their clinical utility is limited. The true measure of leukocyte depletion, and therefore activity during CPB, is a decrease in neutrophil count. Fewer neutrophils in the leukocyte-filtered circuit means that there will be fewer tissue neutrophils after surgery. Consequently, postoperative physiologic indices will be less compromised and the patient will recover expiditiously [46, 53].

Leukocytes

The effects of leukocyte depletion have appropriately focused on changes in the levels of neutrophils, and lymphocyte function and levels have not been scrutinized. CPB is associated with a temporary increase in CD8/CD4 ratio and a decrease in cell-mediated immunity, markers of lymphocyte function [54]. In comparison to neutrophils, these changes are minimal; nevertheless, they are relevant for two reasons. First, the relatively small changes in lymphocyte number reinforce the importance of the neutrophil in mediating reperfusion injury. Second, a further decrease in lymphocyte number because of filter use will compromise host immunity. Therefore, future studies on leukocyte depletion should consider long-term infectious complications as a dependent variable when reporting results.

Another cell of considerable importance in mediating inflammatory stress is the tissue macrophage and blood-borne monocyte. Monocytes circulate in the blood and, in response to chemotactic signals, localize to the site of injury.

When monocytes localize into the inflamed tissue, they differentiate into tissue macrophages. Unlike neutrophils, which are present during the first days of inflammation, macrophages tend to enter tissue later during the inflammatory process. Macrophages phagocytize the remnants of tissue digested by the neutrophils. Although macrophages are predominantly active later in the inflammatory response, they are a rich source of proinflammatory cytokines during the initial injury. They are, in fact, one of the most prolific producers of tumor necrosis factor and interleukins in humans.

The consequences of leukocyte filters on monocyte number and function have not been closely investigated. Monocytes are larger than neutrophils and would easily be trapped in the standard filters used during CPB. Although these cells would be retained in the filter, the small peptides secreted by macrophages would not. In fact, the collection of neutrophils, monocytes and lymphocytes localized in the filter may serve as a nidus of inflammation. All of these cells secrete cytokines, which function in both an autocrine and paracrine manner. Although the monocytes will be isolated from tissue with the use of a filter, their peptide products may still be released into the circulation. These suppositions would be supported by measuring local levels of cytokines both before and immediately after the filter.

Platelets are also invariably trapped in filters but the effects of filter depletion are difficult to separate from those functional changes seen in platelets secondary to bypass. Platelets are cell fragments with granules containing proinflammatory, vasoconstrictive and procoagulant peptides. Release of platelet granule contents is an integral part of the initial inflammatory response. During CPB, circulating platelets are activated and total platelet counts decrease. Moreover, those platelets that are remaining do not function normally. Considering the changes that occur in platelet number and function during bypass, it is difficult to appreciate the difference that leukocyte filtration would have on platelets.

Neutrophil Endothelial Cell Interaction

The mechanism by which neutrophils cause reperfusion injury is fundamentally dependent on the interaction between endothelial cells and neutrophils. Two decades ago the endothelium was considered a passive barrier between the blood and tissues. Investigation of HUVECs has drastically altered this view. It is now recognized that endothelial cells are active and often the principal participants in all types of inflammation and injury.

In the quiescent state, the endothelium secretes substances that maintain an antithrombotic, vasodilated vessel that promotes laminar blood flow. These substances include nitric oxide, adenosine antithrombin III and the arachidonic acid metabolite prostacyclin. This quiescent endothelial cell surface changes

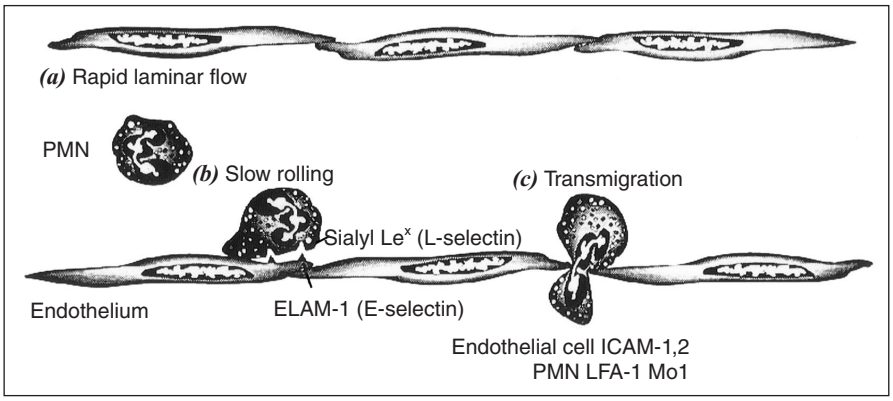


Fig. 3. Neutrophil chemotaxis. Neutrophil chemotaxis and activation is a coordinated multistep process in which neutrophils ultimately transmigrate into the extracellular space and release the contents of their granules producing localized damage.

drastically when the vasculature is exposed to stress. Bacteria, proinflammatory cytokines, trauma and hypoxia are all common clinical scenarios associated with endothelial cell stress and these stimuli lead to endothelial cell activation. Endothelial cell activation is defined as a quantitative change in the surface properties of the endothelium that cumulatively results in the localization of inflammation to the site of injury.

The steps involved in neutrophil chemotaxis and activation at the site of injury are a coordinated multistep process. Neutrophils are recruited from the blood and begin to roll along the endothelial cell surface. This initial rolling is mediated through interactions between constitutively expressed molecules on the leukocyte with cognate counterstructures on the endothelial cell (fig. 3). These molecules are carbohydrate-rich ligands termed selectins. Both E- and P-selectins are expressed on endothelial cells and L-selectin is found on the neutrophil surface. In the unstimulated state, the endothelial cell does not express selectins along the vascular surface, but with stress, P-selectin is released immediately onto the endothelial cell surface from granules in the cytoplasm known as Weibel Palade bodies. E-selectin also appears on the endothelial surface, but its expression requires gene transcription and protein synthesis. The low-affinity binding state resulting from selectin interactions localizes neutrophils to the site of inflammation.

High-affinity bonds between neutrophils and endothelial cells occur subsequent to rolling and are mediated by adhesion molecules. Endothelial adhesion molecules are members of the immunoglobulin gene superfamily and include intracellular adhesion molecule 1 and 2 (ICAM-1/ICAM-2) as well as

platelet endothelial cell adhesion molecule 1 (PECAM 1). The corresponding receptors on neutrophils are termed integrins. Integrins are a heterogeneous family of leukocyte cell surface receptors that are critical to high-affinity neutrophil binding. The neutrophil integrin consists of two subunits CD11b and 18; this complex is also called the MAC-1 receptor. The interaction between endothelial adhesion molecules and neutrophil integrin lead to firm adherence.

After neutrophils have attached to the endothelium, they undergo changes in shape to facilitate transmigration through the endothelial cell barrier. Shape change in neutrophils is a process regulated by local peptides in a paracrine fashion. These changes in neutrophil morphology are not only necessary for transmigration of the cells through the tight intraendothelial junctions but also indicate cellular activation. Transmigration of cells through the endothelial monolayer is controlled and directed by gradients of chemotactic agents. These agents are typically chemokines that form a concentration gradient in the subendothelial matrix. By interacting with G-protein-linked receptors on the neutrophil surface, chemokines actively attract circulating neutrophils into tissue.

Once in tissue, neutrophils release the contents of their granules into the extracellular space. The granule contents digest molecules in the extracellular matrix into amino acids, nucleic acids and simple sugars. In addition, neutrophils are able to produce oxidative damage through the release of myeloperoxidase and the NADPH-oxidase-mediated respiratory burst. The damage produced by neutrophils is typically localized to the tissue and is relatively brief. Within 2 days, neutrophils have been replaced by tissue macrophages, which scavenge the byproducts of degradation resulting from initial neutrophil infiltration. By 1 week, all neutrophil products and cells are undetectable in the affected tissue.

An excellent example of localized neutrophil response occurs in a bacterial abscess. At the site of the abscess, the endothelial cells are activated by endotoxin secreted from the bacterial cell wall. Neutrophils are attracted to the area of infection by activated endothelial cells. Neutrophils infiltrate the tissue, kill the bacteria and break down the bacterial cell products. If the process is successful, the abscess resolves and the tissue remodels with minimal consequences.

At times, the inflammatory process is not localized. This is particularly relevant in two clinical situations, CPB and trauma. In these situations there is generalized endothelial activation caused by circulating mediators that leads to a whole-body inflammatory response. These mediators were discussed in the previous section and include complement, cytokines, thrombin, and circulating oxygen free radicals. Certain vascular beds are more prone to the whole-body inflammatory response, namely the lung and the gastrointestinal tract. The large surface areas of the vascular beds in these organs may predispose them to inflammatory injury or the endothelial cells may be inherently more susceptible to injury.

Reperfusion Injury and Leukocyte Depletion

Strategies to inhibit neutrophil adherence to endothelial cells have shown promise in animal studies, but have been less successful in clinical situations. Cardiovascular surgeons have expressed significant interest in modulating neutrophil adhesion [55–57]. CPB results in an increased expression of L-selectin and CD11b/18 on the surface of neutrophils. This increased adhesiveness of neutrophils may facilitate cell mediated reperfusion injury. In addition to P-selectin, a product of the activated endothelium, is also increased during CPB [58]. Attempts to inhibit L-selectin and P-selectin using monoclonal antibodies have been successful in reducing infiltrating neutrophils in vitro, but the physiologic consequence of the blockade of this low-affinity interaction has been unremarkable [unpubl. data]. To date, there are no clinical trials investigating the specific blockade of selectins.

Blocking antibodies to integrins have also been extensively investigated and these antibodies have been used in several human trials. Initial studies in vitro and in vivo using antibody against CD11b/18 demonstrated remarkable protection against neutrophil-induced I/R injury [59]. These studies were extended into the clinical arena in both trauma patients and cardiac surgery patients [60]. The results of these studies suggest that there is some physiologic benefit to blocking the MAC-1 receptor but the consequences are not dramatic. Other investigators have sought to achieve the same effects by blocking the upregulation of CD11b/18 in animal models [61]. Cardiac surgery seems particularly suited to this type of pharmacologic manipulation because the drug or antibody can be given before the onset of inflammation (CPB). However, the limited successes of these initial experiments and concerns about postoperative infections have discouraged the continued investigation of these interventions.

Among the most potent neutrophil chemotaxins and activators, is a group of chemotactic cytokines called chemokines. Chemokines are a group of short peptides with specific activity for discrete groups of leukocytes. There are two large families of chemokines: the alpha chemokines, which interact primarily with neutrophils, and the beta chemokines, which interact predominantly with monocytes.

The prototypic alpha chemokine, and the first one discovered is IL-8. IL-8 has been characterized and studied extensively in the last two decades. It is produced by many types of cells but primarily has chemotactic activity toward neutrophils. IL-8 binds to heparin residues and concentrates on the subendothelial matrix creating a gradient that attracts neutrophils. In addition, it activates neutrophils through a surface G protein complex that leads to neutrophil shape change, demargination and eventually discharge of both azurophilic and specific granules. There are several other alpha chemokines that have chemotactic activity for neutrophils, including MIP-1α, MIP-2, PF-4, ENA-78, and Gro-α.

Animal studies have demonstrated that blocking the expression of alpha chemokines attenuates reperfusion injury [62]. Blockade of chemokines typically results in a 25–35% decrease in end organ damage. This partial reduction in reperfusion injury is likely related to the enormous redundancy of the chemokines. To some extent they all produce similar effects in vivo. Chemokine inhibition in clinical situations seems promising and in fact, similar blocking antibodies have been used successfully in transplantation to diminish rejection episodes (OKT3, Zenapax). Further animal and human studies are needed to confirm the effectiveness of these therapies in humans.

Attempts to modulate reperfusion injury have tried to decrease the number or function of neutrophils or decrease the generalized cytokinemia that accompanies CPB. CPB affords clinicians and scientists an attractive model in which to modulate inflammation, primarily because cardiac surgery is typically elective and many of the variables involved in the operation can be carefully controlled and monitored. Despite the utility of this model, there have not been dramatic changes in the inflammatory response to bypass with various therapies.

Leukocyte filters have been used clinically to diminish the cell-mediated reperfusion injury caused by CPB. These filters have been used at various times and applied to particular procedures during cardiac operations. For example, filters have been used in line on the arterial side of the extracorporeal circuit [39, 43–46, 63, 64]. They have also been used to deplete blood cardioplegia, cell saver blood, transfused blood, cardiotomy suction blood and preservation solution of leukocytes [14, 65, 66]. The success of these strategies has typically been more apparent in animal experiments, although there is still significant work to be done using these technologies in humans. Blood cell separators have been used during CPB to deplete both leukocytes and platelets [67]. This technology applies a separate low-flow extracorporeal circuit in the venous circulation to deplete blood of leukocytes and platelets. The circuit is run throughout the operation and depleted leukocytes and platelets are returned to the patient at the end of the operation. It appears that the blood cell separator improves some of the adverse effects associated with CPB, but this device has not yet been widely applied. Plasmapheresis before CPB has also used as a leukocyte depletion strategy [68].

The timing of leukocyte depletion is critical to effective results with the filter. Depleting the patient of leukocytes before the institution of bypass avoids contact activation of neutrophils and activation by cytokines generated in the circuit. Replacing the removed leukocytes and platelet products at the end of procedure avoids postoperative infectious concerns. Nevertheless, the use of leukocyte depletion methods does not address the general cytokinemia that accompanies bypass, and this inflammation can contribute to significant organ dysfunction postoperatively.

Hemofiltration both during and after CPB has been used in the pediatric population to diminish cytokinemia associated with CPB [69, 70]. This process removes water and cytokines under a hydrostatic pressure gradient, diminishing both the third space loss as well as inflammatory cytokines during bypass. Despite the enthusiasm for this technology, there are no conclusive data suggesting that it improves long-term function after open-heart operations in children.

Questions still exist about which patients will benefit from modulation of the inflammatory response through pharmacologic (drugs/antibodies), physical (filters) and biomaterial advances. Although some attempts to modulate inflammation, for example leukocyte filters, are thought to be innocuous, the use of these controversial therapies in an era of limited reimbursement is difficult to justify. There may be specific at-risk populations that would benefit from individual (filter alone) or combined (filter + drug) approaches to modulate inflammation. Future studies are required to define this high-risk group and to evaluate the utility of these combined therapies in clinical cardiac surgery, especially since the inflammatory response during CPB is so redundant.

Ultimately I/R injury and inflammation can be entirely circumvented by excluding the bypass circuit. This strategy has gained widespread attention in clinical cardiac surgery and is termed 'off-pump coronary artery bypass' (OP-CAB). Although technically challenging, this approach avoids the systemic inflammatory response of CPB and achieves comparable results to traditional on-pump coronary bypass operations. OP-CAB is the ultimate strategy to abate inflammation associated with I/R injury during CPB because it inherently eliminates the circuit. Not all patients who undergo surgery can be approached using OP-CAB and therefore the inflammation associated with bypass still needs to be addressed. Future strategies should be based on a clear understanding of the molecular basis of the cell-mediated and humoral cascades activated during CPB. An understanding of these events at the molecular level will produce effective strategies to modulate the inflammatory response.

References

1 Kirklin JK: Prospects for understanding and eliminating the deleterious effects of cardiopulmonary bypass (editorial; comment). Ann Thorac Surg 1991;51:529–531.

2 Hearse DJ, et al: Ultrastructural damage associated with reoxygenation of the anoxic myocardium. J Mol Cell Cardiol 1975;7:315–324.

3 Griesmacher A, Windischbauer A, Muller MM: Endothelial cells' responses to hypoxia and reperfusion. Adv Exp Med Biol 1994;370:299–302.

4 Reuter A, Klinger W: The influence of systemic hypoxia and reoxygenation on the glutathione redox system of brain, liver, lung and plasma in newborn rats. Exp Toxicol Pathol 1992;44: 339–343.

5 Rao PS, Cohen MV, Mueller HS: Production of free radicals and lipid peroxides in early experimental myocardial ischemia. J Mol Cell Cardiol 1983;15:713–716.
6 Perez HD, Weksler BB, Goldstein IM: Generation of a chemotactic lipid from a arachidonic acid by exposure to a superoxide-generating system. Inflammation 1980;4:313–328.
7 Buckberg GD: Studies of hypoxemic/reoxygenation injury. I. Linkage between cardiac function and oxidant damage. J Thorac Cardiovasc Surg 1995;110:1164–1170.
8 Zweier JL: Measurement of superoxide-derived free radicals in the reperfused heart. Evidence for a free radical mechanism of reperfusion injury. J Biol Chem 1988;263:1353–1357.
9 Zweier JL, et al: Recombinant superoxide dismutase reduces oxygen free radical concentrations in reperfused myocardium. J Clin Invest 1987;80:1728–1734.
10 Bolli R, et al: Demonstration of free radical generation in 'stunned' myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitrone (published erratum appears in J Clin Invest 1988;82:following 1807). J Clin Invest 1988;82:476–485.
11 Zweier JL, Flaherty JT, Weisfeldt ML: Direct measurement of free radical generation following reperfusion of ischemic myocardium. Proc Natl Acad Sci USA 1987;84:1404–1407.
12 Jurmann MJ, et al: Oxygen-derived free radical scavengers for amelioration of reperfusion damage in heart transplantation. J Thorac Cardiovasc Surg 1988;95:368–377.
13 Bando K, et al: Successful extended hypothermic cardiopulmonary preservation for heart-lung transplantation. J Thorac Cardiovasc Surg 1989;98:137–145, 145–146.
14 Bando K, et al: Twelve-hour cardiopulmonary preservation using donor core cooling, leukocyte depletion, and liposomal superoxide dismutase. J Heart Lung Transplant 1991;10:304–309.
15 Davies GC, Sobel M, Salzman EW: Elevated plasma fibrinopeptide A and thromboxane B_2 levels during cardiopulmonary bypass. Circulation 1980;61:808–814.
16 Kobinia GS, et al: Effect of experimental cardiopulmonary bypass on systemic and transcardiac thromboxane B_2 levels. J Thorac Cardiovasc Surg 1986;91:852–857.
17 Hennein HA, Walenga JM, Bakhos M: Inflammation following cardiopulmonary bypass: The post-pump syndrome; in Pifarre R (ed): Management of Bleeding in Cardiovascular Surgery, Philadelphia, Hanley & Belfus, 1999, pp 357–381.
18 Nelson P, et al: PAF production by endothelial cells: A novel signal for cell-cell interaction; in Dennis EA, Hunter T, Berridge M (eds): Cell Activation and Signal Initiation: Receptor and Phospholipase Control of Inositol Phosphate, PAF, and Eicosanoid Production. New York, Liss, 1989, pp 297–310.
19 Hoshikawa-Fujimura AY, et al: PAF-acether, superoxide anion and beta-glucuronidase as parameters of polymorphonuclear cell activation associated with cardiac surgery and cardiopulmonary bypass. Braz J Med Biol Res 1989;22:1077–1082.
20 Zehr KJ, et al: Platelet activating factor inhibition reduces lung injury after cardiopulmonary bypass [see comments]. Ann Thorac Surg 1995;59:328–335.
21 Morgan EN, et al: Platelet-activating factor acetylhydrolase prevents myocardial ischemia-reperfusion injury. Circulation 1999;100(suppl 19):II365–II368.
22 Loucks EB, Symersky P, Qayumi AK: Platelet-activating factor antagonism: A new concept in the management of regional myocardial ischemia-reperfusion injury. J Invest Surg 1997;10:321–338.
23 Gertler JP, Ocasio VH: Endothelin production by hypoxic human endothelium. J Vasc Surg 1993; 18:178–182, 182–184.
24 Pernow J, Wang QD: Endothelin in myocardial ischaemia and reperfusion. Cardiovasc Res 1997; 33:518–526.
25 Petrone WF, et al: Free radicals and inflammation: Superoxide-dependent activation of a neutrophil chemotactic factor in plasma. Proc Natl Acad Sci USA 1980;77:1159–1163.
26 Bruins P, et al: Activation of the complement system during and after cardiopulmonary bypass surgery: Postsurgery activation involves C-reactive protein and is associated with postoperative arrhythmia. Circulation 1997;96:3542–3548.
27 Amsterdam EA, Rendig SV, Longhurst JC: Contractile actions of C5a on isolated porcine myocardium. Am J Physiol 1992;263:H740–H745.
28 Homeister JW, Satoh P, Lucchesi BR: Effects of complement activation in the isolated heart. Role of the terminal complement components. Circ Res 1992;71:303–319.

29 Stahl GL, Reenstra WR, Frendl G: Complement-mediated loss of endothelium-dependent relaxation of porcine coronary arteries. Role of the terminal membrane attack complex. Circ Res 1995; 76:575–583.
30 Lennon PF, et al: Complement-induced endothelial dysfunction in rabbits: Mechanisms, recovery, and gender differences. Am J Physiol 1996;270:H1924–H1932.
31 Friedman M, et al: Altered beta-adrenergic and cholinergic pulmonary vascular responses after total cardiopulmonary bypass. J Appl Physiol 1995;79:1998–2006.
32 Rinder CS, et al: Blockade of C5a and C5b-9 generation inhibits leukocyte and platelet activation during extracorporeal circulation. J Clin Invest 1995;96:1564–1572.
33 Tofukuji M, et al: Anti-c5a monoclonal antibody reduces cardiopulmonary bypass and cardioplegia-induced coronary endothelial dysfunction. J Thorac Cardiovasc Surg 1998;116:1060–1068.
34 Fitch JC, et al: Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. Circulation 1999;100:2499–2506.
35 Kaplanski G, et al: Thrombin induces endothelial type II activation in vitro: IL-1 and TNF-alpha-independent IL-8 secretion and E-selectin expression. J Immunol 1997;158:5435–5441.
36 Rabiet MJ, Plantier JL, Dejana E: Thrombin-induced endothelial cell dysfunction. Br Med Bull 1994;50:936–945.
37 Kaplanski G, et al: Thrombin-activated human endothelial cells support monocyte adhesion in vitro following expression of intercellular adhesion molecule-1 (ICAM-1; CD54) and vascular cell adhesion molecule-1 (VCAM-1; CD106). Blood 1998;92:1259–1267.
38 Perlmutter DH, et al: Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression. J Clin Invest 1986;78:1349–1354.
39 Johnson D, et al: Depletion of neutrophils by filter during aortocoronary bypass surgery transiently improves postoperative cardiorespiratory status. Chest 1995;107:1253–1259.
40 Knudsen F, Andersen LW: Immunological aspects of cardiopulmonary bypass. J Cardiothorac Anesth 1990;4:245–258.
41 Nilsson L, et al: Endotoxins in cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;100: 777–780.
42 Andersen LW, et al: Presence of circulating endotoxins during cardiac operations. J Thorac Cardiovasc Surg 1987;93:115–119.
43 Lust RM, et al: In-line leukocyte filtration during bypass. Clinical results from a randomized prospective trial. ASAIO J 1996;42:M819–M822.
44 Mihaljevic T, et al: The influence of leukocyte filtration during cardiopulmonary bypass on postoperative lung function. A clinical study. J Thorac Cardiovasc Surg 1995;109:1138–1145.
45 Wilson IC, et al: Temporary leukocyte depletion reduces ventricular dysfunction during prolonged postischemic reperfusion. J Thorac Cardiovasc Surg 1993;106:805–810.
46 Gu YJ, et al: Leukocyte depletion results in improved lung function and reduced inflammatory response after cardiac surgery. J Thorac Cardiovasc Surg 1996;112:494–500.
47 Metchnikoff E, et al: Immunity in Infective Disease. London, Cambridge University Press, 1907.
48 Seghaye MC, et al: Complement, leukocytes, and leukocyte elastase in full-term neonates undergoing cardiac operation. J Thorac Cardiovasc Surg 1994;108:29–36.
49 Butler J, et al: Effect of cardiopulmonary bypass on systemic release of neutrophil elastase and tumor necrosis factor (published erratum appears in J Thorac Cardiovasc Surg 1993;105:1056) (see comments). J Thorac Cardiovasc Surg 1993;105:25–30.
50 Shastri KA, et al: Neutrophil lactoferrin release during open heart surgery is unrelated to complement activation. ASAIO J 1994;40:56–61.
51 Faymonville ME, et al: Myeloperoxidase and elastase as markers of leukocyte activation during cardiopulmonary bypass in humans (see comments). J Thorac Cardiovasc Surg 1991;102: 309–317.
52 Semb AG, et al: Cardiac surgery and distribution of the leukocyte L1 protein-calprotectin. Eur J Cardiothorac Surg 1991;5:363–367.
53 Wilson IC, et al: Leukocyte depletion in a neonatal model of cardiac surgery. Ann Thorac Surg 1993;55:12–19.

54 Nguyen DM, Mulder DS, Shennib H: Effect of cardiopulmonary bypass on circulating lymphocyte function. Ann Thorac Surg 1992;53:611–616.
55 Finn A, Rebuck N, Moat N: Neutrophil activation during cardiopulmonary bypass (letter; comment) (see comments). J Thorac Cardiovasc Surg 1992;104:1746–1748.
56 Dreyer WJ, Smith CW, Entman ML: Reply to: Neutrophil activation during cardiopulmonary bypass (J Thorac Cardiovasc Surg 1992;104:1746–1748) (letter; comment). J Thorac Cardiovasc Surg 1993;105:763.
57 Dreyer WJ, Smith CW, Entman ML: Invited letter concerning: neutrophil activation during cardiopulmonary bypass (letter; comment) (see comments). J Thorac Cardiovasc Surg 1991;102: 318–320.
58 Komai H, Haworth SG: Effect of cardiopulmonary bypass on the circulating level of soluble GMP-140. Ann Thorac Surg 1994;58:478–482.
59 Vedder NB, et al: Inhibition of leukocyte adherence by anti-CD18 monoclonal antibody attenuates reperfusion injury in the rabbit ear. Proc Natl Acad Sci USA 1990;87:2643–2646.
60 Verrier ED, Shen I: Potential role of neutrophil anti-adhesion therapy in myocardial stunning, myocardial infarction, and organ dysfunction after cardiopulmonary bypass. J Card Surg 1993; 8(suppl 2):309–312.
61 Gillinov AM, et al: Inhibition of neutrophil adhesion during cardiopulmonary bypass. Ann Thorac Surg 1994;57:126–133.
62 Sekido N, Mukaida N, Harada A, Nakanishi I, Watanabe Y, Matsushima K: Prevention of lung reperfusion injury in rabbits by a monoclonal antibody against interleukin-8. Nature 1993;365: 654–657.
63 Westlin W, Mullane KM: Alleviation of myocardial stunning by leukocyte and platelet depletion. Circulation 1989;80:1828–1836.
64 Bando K, et al: Leukocyte depletion ameliorates free radical-mediated lung injury after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;99:873–877.
65 van de Watering LM, et al: Beneficial effects of leukocyte depletion of transfused blood on postoperative complications in patients undergoing cardiac surgery: A randomized clinical trial. Circulation 1998;97:562–568.
66 Perttila J, et al: Leucocyte content in blood processed by autotransfusion devices during open-heart surgery. Acta Anaesthesiol Scand 1995;39:445–448.
67 Morioka K, et al: Leukocyte and platelet depletion with a blood cell separator: Effects on lung injury after cardiac surgery with cardiopulmonary bypass (see comments). J Thorac Cardiovasc Surg 1996;111:45–54.
68 Davies GG, et al: Platelet-leukocyte plasmapheresis attenuates the deleterious effects of cardiopulmonary bypass (see comments). Ann Thorac Surg 1992;53:274–277.
69 Elliott MJ: Ultrafiltration and modified ultrafiltration in pediatric open heart operations. Ann Thorac Surg 1993;56:1518–1522.
70 Keenan HT, et al: Pulmonary function after modified venovenous ultrafiltration in infants: A prospective, randomized trial. J Thorac Cardiovasc Surg 2000;119:501–505.

Edward D. Verrier, MD, University of Washington, Department of Surgery,
Division of Cardiothoracic Surgery, 1959 NE Pacific Street, AA110, Box 356310,
Seattle, WA 98195 (USA)
Tel. +1 206 543 3093, Fax +1 206 543 0325, E-Mail edver@u.washington.edu

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 78–88

Mechanism and Strategy of Leukocyte Depletion during Cardiopulmonary Bypass Surgery

Y.J. Gu, A.J. de Vries, P.W. Boonstra, W. Van Oeveren

Cardiothoracic Surgery, Anesthesiology, and Biomedical Engineering
University of Groningen, The Netherlands

Polymorphonuclear leukocytes are known to play a central role in the development of systemic inflammatory response and organ reperfusion injury in cardiac surgical patients operated upon with the use of cardiopulmonary bypass (CPB). For decades since the beginning of CPB surgery, numerous interventions and therapies, such as pharmacological inhibition by corticosteroids and immunological blockage with monoclonal antibodies against leukocyte adhesion, have been launched to diminish the overstimulation of leukocytes induced by the contact of blood with the bypass circuit. However, none of these interventions can be applied without side effects. In the late 1980s and early 1990s, the concept of leukocyte depletion with leukocyte-depleting filters was introduced in an attempt to deplete the majority of circulating leukocytes which are considered to be activated by the CPB circuit. Since then, the application of leukocyte-depleting filters during heart operations has been frequently reported as an alternative and simple therapy in order to minimize the inflammatory response mediated by the activated leukocytes.

Unlike conventional blood filters that are usually screen type filters and depend on pore size, leukocyte-depleting filters are depth filters whose mechanism of filtration is largely dependent on the interaction between leukocytes and the filter material. Complement activation may be involved in leukocyte-material interaction to facilitate leukocyte adhesion to the filter material. Platelets are also known to be trapped in the filter material which could be an important mechanism in leukocyte depletion.

This chapter gives an overview on how leukocytes are trapped inside the leukocyte-depleting filter under different circumstances of blood-material interaction, and describes current and future strategies of leukocyte depletion in CPB surgery.

Historical Aspects

The concept of leukocyte depletion with a leukocyte removal filter was introduced in 1926 by Fleming [1] and Wright [2]. Since then, different types of leukocyte-depleting filters utilizing different natural or synthetic materials have been described. One of the earliest leukocyte-depleting filters was made from medical cotton wool packed as a filter column [3]. These cotton wool filters can remove as many as 95% of leukocytes from whole blood anticoagulated with adenine-citrate-dextrose or heparin. A major disadvantage of the cotton wool filter is the need for a relatively high pressure gradient to let the blood pass through the filter. Thus it is clinically impractical for the filtration of blood directly from a plastic bag during bedside transfusion. Another category of material used for manufacturing the earlier leukocyte-depleting filter is cellulose [4], which was described to have a high efficiency in removing leukocytes.

However, most cellulose products are expensive, and these materials are known to activate the complement system by forming C3 convertase on their surface [5]. Notwithstanding these drawbacks, leukocyte-depleting filters made from cellulose acetate were extensively used in blood banks to prepare leukocyte-poor red cell concentrates with satisfactory performance [6, 7]. Since the late 1980s and early 1990s, polyester leukocyte-depleting filters made from non-woven structure were introduced and immediately gained wide popularity [8, 9]. With its improved flow conditions and low priming volume, polyester filters were used not only in the blood bank but also at the bedside for leukocyte depletion of donor red cell concentrates during transfusion [10, 11].

In 1989 and 1990, animal experiments using these blood bank leukocyte-depleting filters demonstrated reduced myocardial and pulmonary reperfusion injury [12, 13]. These favorable experimental findings led to the early clinical exploration of transfusion filters to deplete autologous leukocytes for cardiac surgery patients and transplanted human hearts [14, 15]. Since 1993, systemic leukocyte depletion utilizing an arterial-line filter has been reported with favorable results [16, 17]. Meanwhile, leukocyte depletion was applied locally for depleting leukocytes during infusion of blood cardioplegia [18, 19]. In 1995, leukocyte depletion of the residual CPB perfusate was found to be a feasible method beneficial to the lungs [20–22]. In 1999, much of the attention devoted to leukocyte depletion was shifted to the reperfusion phase, with a venous

approach starting before reperfusion [23] or arterial filtration commencing at the moment of reperfusion [24, 25]. With an increased understanding of the filtration mechanism and blood-material interaction, combination and further modification of the above-mentioned leukocyte-depleting methods for cardiac surgical patients are now becoming attractive.

Mechanisms of Leukocyte-Depleting Filters

Leukocyte-depleting filters are different from the conventional screen filters. The current leukocyte-depleting filters manufactured either for blood banks or for bedside filtration are classified as 'depth filters', which evolved from the conventional filters called 'screen filters'. Instead of the pore size, a technical qualification widely used in clinical practice, the majority of leukocyte-depleting filters are made of nonwoven fibres, removing leukocytes through several other mechanisms or the combination of mechanisms, rather than by mechanical entrapment only. There are at least four kinds of entrapment of leukocytes which have been described as the mechanisms of the leukocyte-depleting filters. They are: blocking, bridging, interception and adhesion [26]. These mechanisms may play their roles independently or in combination. Blocking occurs when the cell is larger than the diameter of the pore, a mechanism similar to cell entrapment in the screen filter. Bridging means that two or more cells are passing simultaneously through a pole and are blocked as an aggregate. Interception is a process in which cells are mechanically trapped in the dead space around the fibre structure, a mechanism different from blocking which is depending on the pore size. On the contrary, adhesion is an active process which is independent of any mechanical force, such as gravity and flow direction. The mechanism of adhesion is thought to play a much more important role than the other three mechanisms in leukocyte-depleting filters because the size of cells trapped inside the filter is much smaller than the pores of the filter. These large-pore depth filters have the advantage of high blood flow under low pressure and low subsequent blood damage.

Role of Complement Activation in Leukocyte Depletion

Complement components, as well as other substances in plasma, are likely to be involved in the leukocyte adhesion process if adhesion is considered a dominant mechanism of leukocyte removal by the leukocyte-depleting filters [5, 27–31]. The leukocyte surface-adhesive receptors may recognize the degradation products of complement components which are bound to the material surface. On the biomaterial surface, the major activated complement fragment C3b is chemically bound to the surface hydroxyl groups, forming a C3 convertase

by an interaction with other alternative-pathway complement proteins, which augments further C3 cleavage and the subsequent deposition of C3b molecules on the surface. Leukocytes are known to bear the complement receptor type 3 (CR3) which is the receptor for C3b [29, 32]. In a study comparing different types of leukocyte-depleting filters during leukocyte filtration of CPB perfusate [33], increased C3a concentrations, the minor fragment of complement C3 released into plasma after activation, were found in the effluent during filtration with a filter made from cellulose acetate, indicating that the complement cascade was activated and the deposition of its counterpart C3b on the filter surface was conceivable. Complement activation only appeared when fresh whole blood was filtered, whereas no obvious complement activation was found during filtration of banked red blood cell concentrates which are known to have much less active plasma components than fresh whole blood (table 1). With a higher activity of complement, the observed leukocyte-depletion rate in the cellulose acetate filter group was also higher [34], showing that the complement system is involved in leukocyte adherence to the filter material.

Role of Platelets in Leukocyte Depletion

Platelet deposition on the filter material surface has been postulated as a prerequisite for leukocytes to adhere to the filter medium [34, 35]. In a carefully designed laboratory study, leukocyte-depleting filters removed significantly fewer leukocytes from the platelet-depleted buffy-coat or red blood cell concentrates than from control blood products which were not platelet-depleted [34]. When platelets were reloaded into the filter, the leukocyte removal rate recovered and increased with the increased number of platelets loaded to the filter; this positive correlation strongly suggests a role of platelets in leukocyte depletion. Morphological studies showed that platelets had a higher affinity for the filter material than any other type of blood cell. The majority of the deposited platelets were on the top of the filter medium, independent of the filter material (polyester or cellulose acetate). The adhered platelets were morphologically in either a disk form or a fully spread form, functioning as a bridge linking leukocytes to the material surface [35]. For leukocyte depletion of the CPB perfusate, however, we did not find any correlation between platelets and granulocytes which adhered to the filter material [unpubl. obs.].

Although small in size, platelets are known to have a variety of surface adhesive receptors [36, 37], so that they strongly interact with other types of cells including polymorphonuclear leukocytes [38, 39]. This is also true when they are simultaneously interacting with a foreign surface [35, 40]. The entrapment of platelets inside the leukocyte-depleting filters has been a concern for leukocyte depletion in cardiac surgery patients because platelets are essential for effective postoperative hemostasis [41, 42], although simultaneous platelet

Table 1. Plasma concentrations of complement split products and other components in fresh CPB perfusates or in banked RBC concentrates

Parameter	Number	Before filtration	After filtration
Complement C3a, ng/ml			
CPB perfusate	20	1,980 ± 378	2,296 ± 399
RBC concentrates	20	653 ± 181	699 ± 216
Complement C5a, ng/ml			
CPB perfusate	20	12.0 ± 3.0	37.8 ± 13.7
RBC concentrates	20	5.0 ± 0.7	5.0 ± 0.9
Elastase, μg/l			
CPB perfusate	20	278 ± 24	350 ± 26
RBC concentrates	20	249 ± 41	190 ± 44
β-Glucoronidase, AU			
CPB perfusate	20	82 ± 12	86 ± 13
RBC concentrates	20	36 ± 12	57 ± 21
Thromboxane B_2, pg/ml			
CPB perfusate	20	742 ± 116	694 ± 112
RBC concentrates	20	106 ± 22	99 ± 26
Fibrinopeptide A, ng/ml			
CPB perfusate	20	140 ± 14	142 ± 14
RBC concentrates	20	201 ± 64	157 ± 53
Fibrinogen degradation products, ng/ml			
CPB perfusate	20	2,721 ± 974	3,348 ± 1442
RBC concentrates	20	456 ± 94	384 ± 59

Data are expressed as the mean value ± SEM. Concentrations are determined before and after filtration of either 350 ml banked red blood cell (RBC) concentrates or 700 ml CPB perfusates.

depletion by the leukocyte-depleting filter is believed to be beneficial in preventing platelet deposition to the grafted vessel wall during reperfusion [43, 44].

Removal of Activated Leukocytes by Leukocyte-Depleting Filters

Polymorphonuclear leukocytes are involved in initiating an inflammatory response and ischemic and reperfusion injury. Their activated forms are believed to be most harmful in mediating the pathophysiological processes [45, 46]. Because activated leukocytes are more adhesive than nonactivated cells [47, 48], they are more likely to be trapped inside the filter. An early report indicated that 20% of the expression of the leukocyte-associated activation receptors in

circulating blood was reduced after leukocyte depletion from an in vitro simulated extracorporeal circuit [49].

However, there is limited information about the characteristics of the trapped leukocytes with regard to whether the majority of the cells are granulocytes and whether they are activated. These aspects have been addressed recently in our laboratory by studying the trapped cells inside the polyester leukocyte-depleting filters used for inline leukocyte depletion during CPB [50] both morphologically and immunologically. After clinical use, the filters were brought to the laboratory, washed and fixed with paraformaldehyde to prevent disintegration of the cells during further processing. Within the fixed filter material, significantly more granulocytes were deposited in the first blood contact layer than in the middle and the last layer, as identified under light microscopy. The differentiation of the cells indicated that the majority of the band form and the segmented leukocytes, as well as basophils and eosinophils, were deposited in the first blood contact layer of the filter medium. On the contrary, lymphocytes were trapped mostly in the middle layer of the filter. An immunological study with labeled monoclonal antibodies indicated that the majority of granulocytes trapped inside the filter were activated. These morphological and immunological laboratory findings give the impression that the current polyester leukocyte-depleting filters trap more activated granulocytes than a general population of nonactivated leukocytes, creating an ideal situation for cardiac surgical patients whose granulocytes are being activated during CPB.

Strategies of Leukocyte Depletion in Cardiac Surgery

Current Methodology of Leukocyte Depletion

In the past decade, a number of leukocyte-depleting strategies have been introduced in cardiac surgery (fig. 1). The most popular strategy is the combination of a leukocyte-depleting filter with the conventional arterial-line filter to deplete leukocytes at the beginning of perfusion [16, 17]. This is a logic access because of the commonly observed phenomenon that leukocytes are most extensively activated at the beginning of CPB as a result of blood interaction with the foreign material [51, 52]. Another earlier strategy of leukocyte depletion during CPB is to remove leukocytes from blood cardioplegia with a filter installed in the cardioplegic infusion line in case the autologous blood is used for myocardial protection [18, 19]. This local approach is highly efficient in leukocyte depletion although it has little influence on the systemic leukocyte count. A different approach of leukocyte depletion, aiming largely at protecting the lung, was introduced recently to remove leukocytes from the residual heart-lung machine blood at the end of CPB [20, 22]. This access can prevent not only

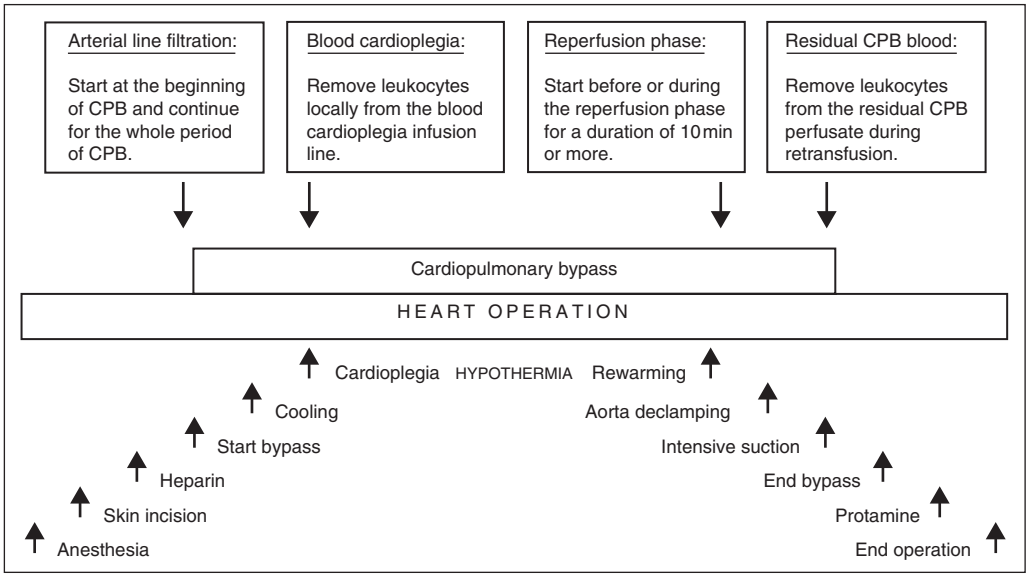


Fig. 1. Leukocyte depletion strategies in cardiac surgery.

the activated leukocytes but also other particles and debris from entering the lungs during retransfusion of the residual CPB perfusate.

More recently, based on a mounting evidence of reperfusion-associated leukocyte sequestration in the heart and lungs [55, 56], the strategy of leukocyte depletion during CPB was shifted to the reperfusion phase. Ideally, the peak of leukocyte depletion in the systemic circulation should be reached before the release of the aortic cross clamp so that the leukocyte entry into the ischemic organ during the reperfusion phase can be maximally reduced. However, in a clinical pilot study involving a 10-min leukocyte depletion before the start of reperfusion, the efficiency of leukocyte filtration was hampered by a parallel increase in circulating leukocytes as a result of rewarming, rendering only 38% of leukocyte reduction instead of the targeted 70% reduction in the systemic circulation [23]. Improved myocardial protection was achieved, however, with a different approach of depleting leukocytes during the initial 15 min of reperfusion with an arterial-line filter [24]. Another report of leukocyte depletion before reperfusion revealed a significant reduction in systemic leukocyte counts, although there was no difference in inflammatory parameters or in clinical outcome in this low-risk patient population with a bypass time of less than 1 h [25]. In longer bypass procedures such as valve replacement, leukocyte depletion after aortic declamping has been reported to result in improved myocardial and

pulmonary function [57]. Since a number of events occur before and during the early phase of reperfusion, including systemic rewarming and intensified cardiotomy suction, searching for an optimal timing and duration of leukocyte depletion is essential for the best performance of the filters.

Timing and Duration of Leukocyte Depletion

As with the arterial-line filtration starting at the beginning of CPB, the leukocyte-depleting filter may catch activated leukocytes by the 'first-pass' effect of blood interaction with the foreign material in the heart-lung machine. This is an obvious advantage of the arterial-line leukocyte filter that has led to its clinical acceptance [58–61]. However, as the arterial-line filter is employed during the entire period of perfusion, saturation of the filter is possible, and low efficiency in reducing the circulating leukocytes was observed [62, 63]. With this general timing policy of leukocyte depletion, there is probably little effective capacity of the filter left as a reserve for depleting leukocytes during the important reperfusion phase. As far as the duration of leukocyte depletion is concerned, there is no consensus so far as to how long it should be. Leukocyte depletion during the whole period of CPB has been associated with increased release of leukocyte enzymes, with trapped leukocytes being a potential source [62, 64]. A shorter period of filtration before or during reperfusion may be insufficient in targeting enough circulating leukocytes as mentioned above.

Conclusion

The technology of leukocyte depletion has been gradually accepted in cardiac surgery as an alternative intervention to reduce the leukocyte-mediated inflammatory response and organ reperfusion injury. Since leukocyte-depleting filters are made from synthetic foreign materials, a better understanding of the filtration mechanism, especially blood interaction with the filter material, is of crucial importance in the development of high efficiency leukocyte-depleting filters for clinical practice. In the near future, new filtration protocols, such as manipulation of timing and duration of leukocyte depletion, or combinations of current methods, are anticipated to reach an optimal leukocyte-depleting strategy for cardiac surgery patients.

References

1 Fleming A: A simple method of removing leukocytes from blood. Br J Exp Pathol 1926;7:281–286.
2 Wright AE: On some new methods, devices and apparatus for the routine and the research laboratory. Lancet 1926;i:4–7.

3 Diepenhorst P, Sprokholt R, Prins HK: Removal of leukocytes from whole blood and erythrocyte suspensions by filtration through cotton wool. I. Filtration technique. Vox Sang 1972;23:308–320.
4 Beutler E, West C, Blume KG: The removal of leukocytes and platelets from whole blood. J Lab Clin Med 1976;328–333.
5 Pangburn MK, Schreiber RD, Muller-Eberhard HJ: C3b deposition during activation of the alternative complement pathway and the effect of deposition on the activating surface. J Immunol 1983;131:1930–1935.
6 Pietersz RNI, Dekker WJA, Reesink HW: A new cellulose acetate filter to remove leukocytes from buffy-coat-poor red cell concentrates. Vox Sang 1989;56:37–39.
7 Steneker I, Biewenga J: Histological and immunohistochemical studies on the preparation of leukocyte-poor red cell concentrates by filtration: The filtration process on cellulose acetate fibers. Vox Sang 1990;58:192–198.
8 Sirchia G, Wenz B, Rebulla P, Parravicini A, Carnelli V, Bertolini F: Removal of white cells from red cells by transfusion through a new filter. Transfusion 1990;30:30–33.
9 Reverberi R, Menini C: Clinical efficacy of five filters specific for leukocyte removal. Vox Sang 1990;58:188–191.
10 Sirchia G, Rebulla P, Parravicini A, Carnelli V, Gianotti GA, Bertolini F: Leukocyte depletion of red cell units at the bedside by transfusion through a new filter. Transfusion 1987;27:402–405.
11 Gu YJ, Obster R, de Haan J, Gallandat Huet RCG, van Oeveren W: Biocompatibility of leukocyte removal filters during bedside leukocyte filtration of red cell concentrates. Transfus Sci 1992;13: 467–472.
12 Breda MA, Drinkwater DC, Laks H, Bhuta S, Corno AF, Davtyan HG, Chang P: Prevention of reperfusion injury in the neonatal heart with leukocyte-depleted blood. J Thorac Cardiovasc Surg 1989;97:654–665.
13 Bando K, Pillai R, Cameron DE, Brawn JD, Winkelstein JA, Hutchins GA, et al: Leukocyte depletion ameliorates free radical-mediated lung injury after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;99:873–877.
14 Gu YJ, Obster R, Gallandat Huet RCG, Eijgelaar A, van Oeveren W: Leukocyte depletion reduces postoperative inflammatory response in patients following open-heart surgery. Intensive Care Med 1992;18:S51.
15 Pearl JM, Drinkwater DC, Laks H, Capouya ER, Gates RN: Leukocyte-depleted reperfusion of transplanted human hearts: A randomized, double-blind clinical trial. J Heart Lung Transplant 1992;11:1082–1092.
16 Palanzo DA, Manley NJ, Montesano RM, Yeisley GL, Gordon D: Clinical evaluation of the LeukoGuard (LG-6) arterial line filter for routine open-heart surgery. Perfusion 1993;8:489–496.
17 Allen SM, Pagano D, Bonser RS: Pall leukocyte depleting filter during cardiopulmonary bypass. Ann Thorac Surg 1994;58:1560.
18 Sawa Y, Matsuda H, Shimazaki Y, Kaneko M, Nishimura M, Amemiya A, et al: Evaluation of leukocyte-depleted terminal blood cardioplegic solution in patients undergoing elective and emergency coronary artery bypass grafting. J Thorac Cardiovasc Surg 1994;108:1125–1131.
19 Pala MG, Paolini G, Paroni R: Myocardial protection with and without leukocyte depletion: A comparative study on the oxidative stress. The Eighth Annual Meeting of the European Association for Cardio-Thoracic Surgery, The Hague, Sept 1994.
20 de Vries AJ, Gu YJ, Boonstra PW, van Oeveren W: Transfusion of leucocyte-depleted heart-lung machine blood improves lung function following cardiopulmonary bypass. Br J Anaesth 1995;74:S54.
21 Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W: Clinical performance of a high-efficiency rapid flow leucocyte removal filter for leucocyte depletion of heparinized cardiopulmonary bypass perfusate. Perfusion 1995;10:425–430.
22 Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W: Leukocyte depletion results in improved lung function and reduced inflammatory response after cardiac surgery. J Thorac Cardiovasc Surg 1996;112:494–500.
23 Gu YJ, de Vries AJ, Vos P, Boonstra PW, van Oeveren W: Leukocyte depletion during cardiac operations: A new approach via the venous side of the cardiopulmonary bypass circuit. Ann Thorac Surg 1999;67:604–609.

24 Matheis G, Scholz M, Gerber J, Abdel-Rahman U, Wimmer-Greinecker G, Moritz A: Leukocyte filtration in the early reperfusion phase on cardiopulmonary bypass reduces myocardial injury. Perfusion 2001;16:43–49.
25 Baksaas ST, Flom-Halvorsen HI, Ovrum E, Videm V, Mollnes TE, Brosstad F, Svennevig JL: Leucocyte filtration during cardiopulmonary reperfusion in coronary artery bypass surgery. Perfusion 1999;14:107–177.
26 Bruil A: Leukocyte filtration: Filtration mechanisms and materials design; thesis. Twente, 1993; pp 8–9.
27 Snyder EL, Root RK, McLeod B, Dalmasso: Activation of complement by blood transfusion filters. Vox Sang 1983;45:288–293.
28 Lang EV, Lang JH, Lasser EC: Adherence of granulocytes to nylon fibers. Evidence for a plasma granulocyte adherence factor. Thromb Res 1988;50:243–248.
29 Takemoto Y, Matsuda T, Kishimoto T, Maekawa M, Akutsu T: Molecular understanding of cellular adhesion on artificial surface. Trans Am Soc Artif Intern Organs 1989;35:354–356.
30 Chenoweth DE: Complement activation during hemodialysis: Clinical observations, proposed mechanisms, and theoretical implications. Artif Organs 1984;8:281–287.
31 van Oeveren W: Leukocyte and platelet activation during extracorporeal circulation. Cells Materials 1994;4:187–195.
32 Gu YJ, van Oeveren W, Boonstra PW, de Haan J, Wildevuur CR: Leukocyte activation with increased membrane expression of CR3 receptors induced by cardiopulmonary bypass. Ann Thorac Surg 1992;53:839–844.
33 Gu YJ, van Oeveren W: Activation of plasma components by leukocyte removal filters. ASAIO J 1994;40:M598–601.
34 Steneker I, Prins HK, Florie M, Loos JA, Biewenga J: Mechanisms of white cell reduction in red cell concentrates by filtration: The effect of the cellular composition of the red cell concentrates. Transfusion 1993;33:42–50.
35 Steneker I, van Luyn MJA, van Wachen PB, Biewenga J: Electron microscopic examination of white cell reduction by four white cell-reduction filters. Transfusion 1992;32:450–457.
36 George JN, Pickett EB, Saucerman S, McEver RP, Kunicki TJ, Kieffer N, Newman PJ: Platelet surface glycoproteins. Studies on resting and activated platelets and platelet membrane microparticles in normal subjects and observations in patients during adult respiratory distress syndrome and cardiac surgery. J Clin Invest 1986;78:340–348.
37 Sakariassen KS, Nievelstein PF, Coller BR, Sixma JJ: The role of platelet membrane glycoproteins Ib and IIb-IIIa in platelet adherence to human artery subendothelium. Br J Haematol 1986;63:681–691.
38 Jungi TW, Spycher MO, Nydegger UE, Barandun S: Platelet-leukocyte interaction: Selective binding of thrombin-stimulated platelets to human monocytes, polymorphonuclear leukocytes, and related cell lines. Blood 1986;67:629–636.
39 Rinder HM, Bonan J, Rinder CS, Ault KA, Smith BR: Dynamics of leukocyte-platelet adhesion in whole blood. Blood 1991;78:173–176.
40 Morley DJ, Feuerstein IA: Adhesion of polymorphonuclear leukocytes to protein-coated and platelet adherent surfaces. Thromb Haemost 1989;62:1023–1028.
41 Boonstra PW, van Imboff GW, Eysman L, Kootstra GJ, Homan van der Heide JN, Karliczek GF, Wildevuur Ch RH: Reduced platelet activation and improved hemostasis after controlled cardiotomy suction during clinical membrane oxygenator perfusions. J Thorac Cardiovasc Surg 1985;89:900–906.
42 Woodman RC, Harker LA: Bleeding complications associated with cardiopulmonary bypass Blood 1990;76:1680–1697.
43 Feinberg H, Rosenbaum DS, Levitsky S, Silverman NA, Kohler J, LeBreton G: Platelet deposition after surgically induced myocardial ischemia: An etiologic factor for reperfusion injury. J Thorac Cardiovasc Surg 1982;84:815–822.
44 Teoh K, Christakis G, Weisel R, et al: Prevention of myocardial platelet deposition and thromboxane release with dipyridamole. Circulation 1986;74(suppl III):145–152.
45 Arnaout MA, Hakim RM, Todd RF III, Dana N, Colten HR: Increased expression of an adhesion-promoting surface glycoprotein in the granulocytopenia of hemodialysis. N Engl J Med 1985; 312:457–462.

46 Patarroyo M, Makgoba MW: Leucocyte adhesion to cells in immune and inflammatory responses. Lancet 1989;ii:1139–1142.
47 Berger M, O'Shea J, Cross AS, Folks TM, Chused TM, Brown EJ, Frank MM. Human neutrophils increase expression of C3bi as well as C3b receptors upon activation. J Clin Invest 1984;74: 1566–1571.
48 Zimmerman GA, Remzetti AD, Hill HR: Functional and metabolic activity of granulocytes from patients with adult respiratory distress syndrome: Evidence for activated neutrophils in the pulmonary circulation. Am Rev Respir Dis 1983;127:290.
49 Thurlow PJ, Doolan L, Sharp R, Sullivan M, Smith B: Studies of the effect of Pall leucocyte filters LG6 and AV6 in an in vitro simulated extracorporeal circulatory system. Perfusion 1995;10:291–300.
50 Smit JJJ, de Vries AJ, Gu YJ, van Oeveren W: Filtration of activated granulocytes during cardiopulmonary bypass surgery: A morphological and immunological study to characterize the trapped leukocytes. J Lab Clin Med 2000;135:238–246.
51 Hammerschmidt DE, Stroncek DF, Bowers TK, et al: Complement activation and neutropenia occurring during cardiopulmonary bypass. J Thorac Cardiovasc Surg 1981;81:370–377.
52 van Oeveren W, Wildevuur Ch RH: Blood compatibility of cardiopulmonary bypass circuits. Perfusion 1987;2:237–244.
53 Royston D, Fleming JS, Desai JB, Westaby S, Taylor KM: Increased production of peroxidation products associated with cardiac operations. Evidence for free radical generation. J Thorac Cardiovasc Surg 1986;91:759–766.
54 Gu YJ, Wang YS, Chiang BY, Gao XD, Ye CX, Wildevuur Ch RH: Membrane oxygenator prevents lung reperfusion injury in canine cardiopulmonary bypass. Ann Thorac Surg 1991;51:573–578.
55 Semb A, Forsdahl K, Vaage J: Granulocyte and eicosanoid gradients across the coronary circulation during myocardial reperfusion in cardiac surgery. Eur J Cardiothorac Surg 1990;4:543–548.
56 Elgebaly SA, Hahsmi FH, Houser SL, Allam ME, Doyle K: Cardiac-derived neutrophil chemotactic factors: Detection in coronary sinus effluents of patients undergoing myocardial revascularization. J Thorac Cardiovasc Surg 1992;103:952–959.
57 Hachida M, Hanayama N, Okamura T, Akasawa T, Maeda T, Bonkohara Y, et al: The role of leukocyte depletion in reducing injury to myocardium and lung during cardiopulmonary bypass. ASAIO J 1995;41:M291–M294.
58 Hart S, Roe JA: Leucocyte depleting filters and cardiothoracic surgery. Perfusion 1993;8:477–482.
59 Allen SM: Leucocyte depletion in cardiothoracic surgery. Perfusion 1996;11:270–277.
60 Allen S, Lloyd G, Butz H: Leucocytes and the systemic inflammatory response. J Anästh Intens 1996;5:S38–S57.
61 Gott JP, Cooper WA, Schmidt FE Jr, Brown WM III, Wright CE, Merlino JD, Fortenbery JD, Clark WS, Guyton RA: Modifying risk for extracorporeal circulation: Trial of four anti-inflammatory strategies. Ann Thorac Surg 1998;66:747–754.
62 Milhaljevic T, Tönz M, von Segesser LK, Pasic M, Grob P, Fehr J, et al: The influence of leukocyte filtration during cardiopulmonary bypass on postoperative lung function: A clinical study. J Thorac Cardiovasc Surg 1995;109:1138–1145.
63 Bolling KS, Halldorsson A, Allen BS, Rahman S, Wang T, Kronon M, Feinberg H: Prevention of the hypoxic reoxygenation injury with the use of a leukocyte-depleting filter. J Thorac Cardiovasc Surg 1997;113:1081–1089.
64 Mair P, Hoermann C, Mair J, Margreiter J, Puschendorf B, Balogh D: Effects of a leucocyte depleting arterial line filter on perioperative proteolytic enzyme and oxygen free radical release in patients undergoing aortocoronary bypass surgery. Acta Anaesthesiol Scand 1999;43:452–457.

Dr. Y. John Gu, MD, PhD, Blood Interaction Research, Cardiothoracic Surgery,
University Hospital Groningen, Hanzeplein 1, NL–9700 RB Groningen (The Netherlands)
Tel. +31 50 363 3127, Fax +31 50 363 3106, E-Mail y.j.gu@med.rug.nl

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 89–100

Leukocyte Filters in Perfusion

David A. Palanzo

Lehigh Valley Hospital, Allentown, Pa., USA

Complications of cardiopulmonary bypass (CPB) include an inflammation-like reaction which is characterized by increased capillary permeability, increased interstitial fluid accumulation, fever and multiple organ dysfunction. These sequelae have been collectively referred to as postpump syndrome, postperfusion syndrome and more recently as systemic inflammatory response syndrome. Pulmonary edema, increased oxygen free radical activity and vasoconstriction which contribute to lung injury during bypass and the reperfusion period have been attributed to the role of circulating leukocytes.

Early work done on animal models demonstrated that by removing circulating leukocytes there was a reduction in pulmonary injury and myocardial ischemic injury following insult [1–3]. From the mid 1980s to the early 1990s, there were many studies involving heart and lung transplantation models with leukocyte depletion [4–13]. Favorable results from these investigations included reduced myocardial damage after preservation, reduction in free radical generation with improved pulmonary function, significant improvement in PaO_2, and improvement in stroke work, cardiac output, left ventricular stroke work index and myocardial blood flow.

The first human studies demonstrated benefits from the reperfusion of leukocyte-depleted blood using transfusion filters. Gu et al. [14] showed a reduction in postoperative inflammatory response following coronary artery bypass grafting when the residual CPB circuit volume was leuko-depleted prior to retransfusion. Pearl et al. [15] showed that leukocyte depleted reperfusion following cardiac transplantation decreased the biochemical evidence of reperfusion injury.

In 1991 a specific leukocyte-depleting arterial line filter for CPB was introduced into the marketplace. Initial in vitro studies with bovine and human blood showed a 70% neutrophil depletion rate with selective removal of activated

neutrophils [16–18]. From these positive results and the previous research demonstrating the advantage of leukodepleting the blood prior to the reperfusion period, we decided to conduct our own clinical evaluation of the Pall LG-6 arterial line filter (Pall Biomedical Products Co., East Hills, N.Y., USA) for routine open-heart surgery cases [19].

Clinical Evaluation

To evaluate the Pall LG-6 arterial line filter, we wanted to compare it against a similar filter. We chose the Pall EC PLUS filter because the two filters are similar in design, both consisting of an automatic venting mechanism for gaseous microemboli and a 40-μm woven polyester screen. Where they differ is that the LG-6 contains a polyester fiber matrix, which has been physically altered to attract white cells located upstream of the filter element.

Thirty-six patients undergoing surgery for coronary artery disease or aortic valvular disease were randomly divided into two equal groups. Each patient received the same CPB circuit except for the arterial line filter. All patients were maintained at a maximum blood flow rate of 2.4 liters/min/m^2 and systemically cooled to 28 °C.

The investigation was limited to patients with normal preoperative lung function so preoperative arterial blood gases, pulmonary function studies and chest X-rays were evaluated on all patients. Patient population variables were collected on all patients preoperatively to ensure that the study patients were all one population prior to surgery. Intraoperative and postoperative data were collected on all patients and compared between the two groups for statistically significant differences.

The patients were weaned from the ventilator using arterial blood gas results. Blood gases were drawn 30 min after every ventilator change and evaluated for further change or extubation. Extubation criteria included that the patient be awake, hemodynamically stable, have an arterial pO_2 of 80 mm Hg or greater on 50% inspired oxygen and have a negative inspiratory flow of less than –20 mm Hg and an expiratory vital capacity of at least 15 cm^3/kg on weaning spirometry. Upon extubation, each patient was placed on a 60% aerosol mask and an arterial blood gas was run 30 min later.

No statistical differences were observed between the two groups for any of the preoperative variables including pulmonary function (table 1). The techniques and equipment used in the operating room and all postoperative care were the same for all patients except that some of the patients received a leukocyte-depleting arterial line filter during CPB. Since all of the patients can be considered the same population prior to surgery, any significant differences

Table 1. Clinical evaluation of leukoguard (LG-6) arterial line filter

	EC PLUS group	LG-6 group	p
Patient population variables			
Number	18	18	
Age, years	66.3 ± 7.7	62.5 ± 11.1	NS
Body surface area, m^2	1.96 ± 0.22	1.98 ± 0.25	NS
Bypass time, min	99.9 ± 30.2	107.7 ± 28.9	NS
Aortic cross clamp time, min	47.7 ± 17.6	54.3 ± 22.4	NS
Circulating formed elements			
Preoperative hemoglobin, g/dl	13.3 ± 1.1	13.4 ± 1.6	NS
Immediate postoperative hemoglobin	10.7 ± 0.9	10.7 ± 0.9	NS
Postoperative hemoglobin at 24 h	9.7 ± 1.2	10.3 ± 1.2	NS
Platelet drop, %			
Preoperative to immediately postoperative	28.3 ± 13.0	29.0 ± 20.0	NS
Preoperative to postoperative at 4 h	25.0 ± 13.7	23.6 ± 17.5	NS
White blood cell count, $\times 10^3/mm^3$			
Preoperation	7.6 ± 1.7	6.8 ± 1.2	NS
Immediately postoperative	13.4 ± 4.8	10.8 ± 4.4	0.09
Postoperative at 24 h	12.4 ± 3.3	10.1 ± 3.2	0.05
Postoperative variables			
24-hour chest tube drainage, ml	657.3 ± 241.6	682.4 ± 257.0	NS
24-hour urine output, liters	3.8 ± 1.2	3.0 ± 1.6	NS
Amount of packed red blood cells given during first 24 h, units	0.83 ± 1.01	0.88 ± 1.08	NS
Ventilator hours[a]	13.3 ± 5.0	9.2 ± 2.1	0.003
Hours in intensive care unit[a]	53.9 ± 27.8	48.3 ± 19.4	NS

Values are expressed as mean ± standard deviation; NS = not significant. Due to the small number a p value of 0.04 was considered significant.

[a] Study was conducted prior to initiation of fast-track anesthesia techniques.

in postoperative variables could be directly related to the type of arterial line filter used.

There were no differences in hemoglobin levels, chest tube drainage, 24-hour urine output, blood and blood product usage and postoperative chest X-rays. There were also no differences observed in the percentage of platelet count drop comparing preoperative values with immediately postoperation and 4 h postoperation. The platelet count drops observed were similar to the results found in vitro by Gourlay et al. [16].

White blood cell counts showed nearly, statistically significant differences postoperatively with the LG-6 group having the lower number of circulating

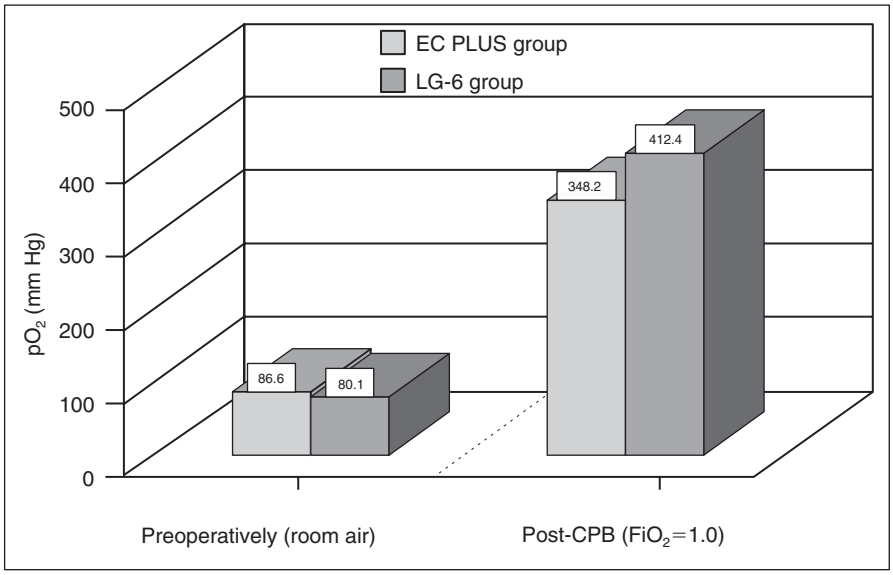


Fig. 1. Pulmonary function. There is a statistically significant difference ($p = 0.02$) between the means of the two groups for the post-CPB pO_2 values.

white blood cells. Even though the white blood cell counts were lower for the LG-6 patients, they were still elevated compared to preoperative levels, demonstrating the characteristic leukocytosis stimulated by CPB. There were no postoperative infections observed in either group.

Two complications attributed to postperfusion syndrome are increased systemic and pulmonary vascular resistance and unexplained elevations in body temperature postoperatively. There were no differences observed in vascular resistance between the groups but the patients who received the EC PLUS filter required higher levels of sodium nitroprusside for longer periods of time than did the LG-6 patients to keep their systemic vascular resistance and pulmonary artery pressures within the normal range. Two patients in the EC PLUS group had unexplained elevated body temperatures during the first 24 h postoperation. These patients had negative blood cultures and their body temperature returned to normal on the second postoperative day.

There was a statistically significant difference between the groups when comparing the immediately post-CPB pO_2s on a fixed FiO_2 of 1.0 (fig. 1). The LG-6 patients had higher values than the EC PLUS patients. The LG-6 patients also required less hours on the ventilator than did the EC PLUS patients. (This study was conducted prior to the initiation of fast-track anesthesia techniques.)

Total postoperative care charges from the day of surgery to discharge were calculated for all patients. The LG-6 group had a mean savings of USD 2,892 in comparison to the EC PLUS group.

Follow-Up Study

As a follow-up investigation, we conducted a retrospective analysis of 350 patients undergoing open-heart surgery over a 5-month period to evaluate the effectiveness of leuko depletion during CPB [20]. From this group we were able to match 120 pairs of patients. Each pair was matched for surgeon, bypass time, cross clamp time, operative procedure and preoperative condition. All patients received the same CPB circuit and conduct of perfusion except that one group of patients received the LG-6 leukodepleting arterial line filter and the other group received a conventional filter (ARS-40, Electromedics, Inc., Englewood, Colo., USA or No. 1362, Medtronic Blood Systems, Inc., Anaheim, Calif., USA).

To ensure that all patients were the same population prior to surgery, patient population variables of gender, age, body surface area, hematocrit and white blood cell count were collected and compared on all patients.

The patients were weaned from the ventilator based on arterial blood gas results and the extubation criteria described previously. To normalize for the differing effects of anesthesia on each patient, the times required for the patients arterial blood gases to reach the extubation criteria were compared for all patients studied.

No differences were found when comparing the preoperative patient population variables and the hematological variables (table 2). The white blood cell counts immediately after the operation and at 4 h postoperatively were lower in the LG-6 patients, though not significantly, demonstrating the effects of leukocyte depletion while on CPB (fig. 2).

Statistically significant differences were noted for the time required to reach the extubation criteria (5.6 $\pm$ 2.7 vs. 7.2 $\pm$ 4.4 h) and the ventilator hours (11.0 $\pm$ 4.1 vs. 15.2 $\pm$ 6.3 h), the time required by the LG-6 group being shorter than that required by the nondepleted group. The postoperative length of stay was also shorter for the LG-6 group (1.4 days). When comparing the postoperative care charges, there was a mean savings of USD 1,942 for the LG-6 group in comparison with the nondepleted group.

Discussion

Most clinical studies of leukocyte depletion have demonstrated few differences in the white blood cell counts during CPB with or without the use of

Table 2. Follow-up investigation

	LG-6 group	Conventional group	p
Patient population variables			
Age, years	63.9 ± 11.3	66.2 ± 9.7	NS
Gender			
Male	84 (70%)	84 (70%)	NS
Female	36 (30%)	36 (30%)	NS
Body surface area, m^2	1.89 ± 0.22	1.91 ± 0.19	NS
Bypass time, min	131 ± 40	131 ± 38	NS
Aortic cross clamp time, min	73 ± 27	75 ± 27	NS
Hematocrit, %			
Preoperatively	40.2 ± 3.7	38.4 ± 4.5	NS
Immediately postoperation	31.8 ± 4.1	30.1 ± 4.6	NS
24 h postoperatively	29.3 ± 3.4	29.0 ± 4.6	NS
Postoperative variables			
Ventilator hours[a]	11.0 ± 4.1	15.2 ± 6.3	<0.05
Time required to reach extubation criteria, h[a]	5.6 ± 2.7	7.2 ± 4.4	<0.05
Postoperative length of stay, days[a]	8.2 ± 2.5	9.6 ± 4.6	<0.05

Values are expressed as mean standard deviation; NS = not significant.
[a] Study was conducted prior to initiation of fast-track anesthesia techniques.

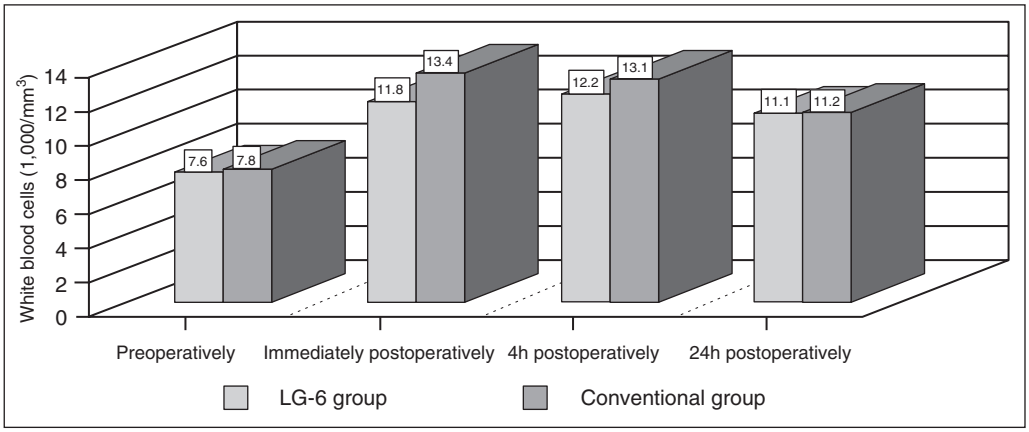


Fig. 2. White blood cell counts. The leukocyte depletion characteristics of the LG-6 arterial line filter are demonstrated in this figure.

Table 3. Early clinical evaluations of the leukocyte-depleting arterial line filter (1993–1995)

Study	Results
Palanzo et al. [19]	Higher PaO_2 immediately post-CPB, less time on ventilator and in ICU
Allen et al. [20]	Postbypass leukocytosis attenuation
Al-Ebrahim and Shaffei [21]	No effect on time on ventilator or white blood cell count
Palanzo et al. [19]	Less time on ventilator and in hospital
Coleman and Demastrie [23]	Need for home supplemental oxygen was reduced
Johnson et al. [24]	Higher PaO_2 in first 4 h, reduction of leukocytes at 4 h postoperatively
Mihaljevic et al. [25]	Increase in elastase in leukocyte depleted group, no difference in lung function
Hachida et al. [26]	Improved pulmonary index at 3 and 6 h postoperatively, lower CK-MB levels at 3 and 6 h postoperatively

leukocyte-depleting filters. Despite these results, some clinical investigations have shown a number of beneficial effects from arterial line leukodepleting on postoperative lung function [19–26] (table 3). Hachida et al. [26] noted a significant decrease in CPK-MB levels in the leukocyte-depleted group at 3 and 6 h postoperatively. Recent evaluations of leukocyte depletion have shown significant differences in postoperative lung and myocardial function [27–34] (table 4). The results of a study by Gu et al. [27] suggest that leukocyte depletion of the residual pump volume improves postoperative lung gas exchange function. Di Salvo et al. [29] observed that patients who were leukodepleted on CPB had lower troponin T and CPK-MB values and less oxidated stress on their myocardium than did the nonfiltered patients. Allen et al. [30] demonstrated that oxygen free radical production was reduced in infants who were leuko-depleted on bypass. In a study by Gott et al. [31] low-risk patients who were leukocyte-depleted during CPB had a length of stay reduced by 1 day and a decrease in their mean hospital charges by USD 2,000 to 6,000. These results are very similar to our original investigation of the LG-6 filter.

Leukocyte depletion of blood cardioplegia has also demonstrated favorable results [35–39] (table 5). Myocardial protection in patients who received leukocyte-depleted blood cardioplegia was observed with a reduction in CPK-MB levels, reduction in inotropic requirements, improved cardiac output, reduced arrhythmias and reduced clinical and biochemical indexes of myocardial reperfusion injury.

Table 4. Recent evaluations of leukocyte depletion (1996–1999)

Study	Results
Gu et al. [27]	Leukocyte depletion of residual pump blood improves postoperative lung gas exchange function
Bolling et al. [28]	Prevents injury to hypoxic heart caused by oxygen radicals, improves myocardial and pulmonary function (piglets)
Di Salvo et al. [29]	Lower troponin T and CPK-MB values, less oxidated stress on the myocardium
Allen et al. [30]	Oxygen free radical production reduced in infants
Gott et al. [31]	Reduced length of stay by one day and mean charges by USD 2,000 to 6,000 in low-risk patients
Zhang et al. [32]	Provided myocardial protection and afforded superior postoperative myocardial contractility (swine model)
Hurst et al. [33]	Filter selectively depletes activated neutrophils
Sheppard et al. [34]	Filter selectively removes activated leukocytes from circulating blood
Baksaas et al. [44]	Use of filter during reperfusion period significantly reduced the number of circulating leukocytes
Matheis et al. [45]	Use of filter prior to removal of aortic cross clamp through initial 15 min of reperfusion reduced tropinin T values

Table 5. Leukocyte-depleted blood cardioplegia

Study	Results
Sawa et al. [35]	Reduction in CPK-MB levels and inotropic requirements
Pala et al. [36]	Limited the oxidative stress following reperfusion in patients with left ventricular dysfunction
Ichihara et al. [37]	Reduced lipid peroxide, elastase and CPK-MB levels
Schmidt et al. [38]	Attenuated leukocyte-mediated endothelial reperfusion injury
Palatianos et al. [39]	Reduces clinical and biochemical indexes of myocardial reperfusion injury

Even though most of the clinical studies demonstrated nonsignificant differences in leukocyte counts and circulating elastase levels during and post-CPB, these same investigations observed marked improvements in postoperative lung function and myocardial function. This may be due to the mechanisms involved in leukocyte-mediated tissue damage. Activated polymorphonuclear neutrophils release proteolytic enzymes in their active forms. These enzymes are quickly neutralized by antiproteases that are circulating within the plasma in high concentrations. Weiss [40] hypothesized that the superoxides that are also

released by the neutrophils inactivate the antiproteases. One example is elastase which is neutralized by alpha-1-antiprotease. In the presence of superoxides, which inactivate alpha-1-antiproteases, elastase was measured at almost 2,000 times the normal value. This enables elastase to have more time to cause damage. The important thing is that this entire sequence must occur within close proximity of the target tissue, which in our case are the lung tissue and myocardium during reperfusion. Even though the neutrophils are releasing their granular components when activated while on CPB, some are now sequestered within the filter matrix far upstream from the target tissues. By the time these components reach the lungs and myocardium, they are bound to plasma proteins and cannot cause the same amount of damage as they would if released directly at the tissue level.

Leukocytes, especially polymorphonuclear neutrophils, play a key role in the systemic inflammatory response that is induced by bypass. Selectively filtering these leukocytes is a simple method employed to help suppress this reaction. Other techniques to blunt the effects of activated neutrophils are being investigated and show promise, but more work needs to be done before these methods are available for routine clinical CPB.

Until new techniques or devices replace the leukocyte-depleting arterial line filter, it is still the best defense that we currently have at our disposal. Refinements in its properties and use are being addressed. The leukocytosis seen during CPB results in an enormous release of leukocytes from the third space. The current filter should be improved to be more efficient at removing a larger amount of circulating leukocytes while reducing the risk of filter obstruction and resistance to flow while on CPB. Smit et al. [41] have recently investigated the efficiency and safety of leukocyte filtration using a new large-capacity leukocyte filter. This study demonstrated that 75% of leukocytes entering the filter were removed by the filter, but flow obstruction by clotting is still an important issue of safety involved in the filtration of large numbers of leukocytes. One method employed to avoid the risk of arterial line filter obstruction is to leukodeplete through the venous side of the bypass circuit [42].

Another area of current investigation is the proper timing for leukocyte removal [43]. Baksaas et al. [44] demonstrated that the use of leukocyte depletion filters during the reperfusion period of elective coronary artery bypass surgery significantly reduced the number of circulating leukocytes. Matheis et al. [45] investigated the use of a leukocyte-depleting arterial line filter prior to removal of the aortic cross clamp through the initial 15 min of reperfusion in low risk patients. Patients who were leukodepleted had lower troponin T values, which indicates improved myocardial protection, and did not require any epinephrine or dobutamine hydrochloride. Their conclusion was that the demonstrated clinical and biochemical benefits justify the routine use of a leukocyte filter.

Smit et al. [46] observed that rather than removing leukocytes at random, a leukocyte filter made of nonwoven polyester material removes activated granulocytes. Studies by Hurst et al. [33] and Sheppard et al. [34] have also demonstrated that the filters selectively remove activated neutrophils. Since patients on CPB have activated granulocytes, this is the type of filter suitable for use. The authors concluded that a better functional assessment for the leukocyte removal devices would be to measure the activated granulocytes instead of total leukocyte counts. If more studies evaluated the removal of activated granulocytes when using a leukocyte depletion filter, there may be more of a correlation between improved postoperative lung and myocardial function and lower counts.

Continued study and investigation with leukocyte depletion need to be performed to refine the technique to obtain optimal results. More definitive studies investigating biochemical and long-term postoperative histological parameters should be done to evaluate any hidden risks or benefits of leukocyte depletion while on CPB. Do patients that were leukodepleted while on bypass tend to be less likely to become patients in the future?

References

1 Heflin AC, Brigham KL: Prevention by granulocyte depletion of increased vascular permeability in sheep following endotoxemia. J Clin Invest 1981;68:1253–1260.
2 Barrie PS, Tahamount MV, Mailk AB: Prevention of increased pulmonary vascular permeability after pancreatitis by granulocyte depletion in sheep. Am Rev Respir Dis 1982;126:904–908.
3 Romson JL, Hook BG, Kumkel SL, Abrams GD, Schork MA, Lucchesi BR: Reduction of the extent of myocardial ischemic injury by neutrophil depletion in the dog. Circulation 1983;67:1016–1023.
4 Breda MA, Hall TS, Stuart RS, et al: Twenty-four hour lung preservation by hypothermia and leukocyte depletion. J Heart Transplant 1985;4:325–329.
5 Hall TS, Breda MA, Baumgartner WA, et al: The role of leukocyte depletion in reducing injury to the lung after hypothermic ischemia. Curr Surg 1987;44:137–139.
6 Breda MA, Drinkwater DC, Laks H, et al: Prevention of reperfusion injury in the neonatal heart with leukocyte depleted blood. J Thorac Cardiovasc Surg 1989;97:654–665.
7 Pillai R, Bando K, Schueler S, Zebley M, Reiz BA, Baumgartner WA: Leukocyte depletion results in excellent heart-lung function after 12 hours of storage. Ann Thorac Surg 1990;50:211–214.
8 Schueler S, Hatanaka M, Bando K, et al: Twenty-four hour lung preservation with donor core-cooling and leukocyte depletion in a bilateral lung transplant model. Surg Forum 1990;41:405–407.
9 Bando K, Pillai R, Cameron DE, et al: Leukocyte depletion ameliorates free-radical mediated lung injury after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;99:873–877.
10 Bando K, Schueler S, Cameron DE, et al: Twelve hour cardiopulmonary preservation using donor core-cooling, leukocyte depletion and liposomal superoxide dismutase. J Heart Lung Transplant 1991;10:304–309.
11 Fukishima N, Skirakura R, Nakata S, et al: Effects of terminal cardioplegia with leukocyte depleted blood on heart grafts preserved for 24 hours. J Heart Lung Transplant 1992;11:676–682.
12 Kawata H, Sawatari K, Mayer JE: Evidence for the role of neutrophils in reperfusion injury after cold cardioplegic ischemia in neonatal lambs. J Thorac Cardiovasc Surg 1992;103:908–918.
13 Byrne JG, Appleyard RF, Lee CC, et al: Controlled reperfusion of the regionally ischemic myocardium with leukocyte depleted blood. J Thorac Cardiovasc Surg 1992;103:66–72.

14 Gu YJ, Obster R, Gallandat Huet RCG, Eijgelaar A, van Oeveren W: Leukocyte depletion reduces the postoperative inflammatory response in patients following open heart surgery. Intensive Care Med 1992;18(suppl 2):S51.
15 Pearl J, Drinkwater DC, Laks H, Caponoya ER, Gates RN: Leukocyte-depleted preperfusion of transplanted human hearts. A randomised, double-blind clinical trial. J Heart Lung Transplant 1992;11:1082–1092.
16 Gourlay T, Fleming J, Taylor KM: Laboratory evaluation of the Pall LG-6 leukocyte depleting arterial line filter. Perfusion 1992;7:131–140.
17 Gourlay T, Fleming J, Taylor KM: The effects of pulsatile flow on the leukocyte depleting qualities of the LG-6 leukocyte depleting arterial line filter. Perfusion 1992;7:227–232.
18 Thurlow PJ, Doolan L, Sharp R, Sullivan M, Smith B: Studies of the effect of Pall leukocyte filters LG6 and AV6 in an in vitro simulated extracorporeal circulatory system. Perfusion 1995;10: 291–300.
19 Palanzo DA, Manley NJ, Montesano RM, Yeisley GL, Gordon D: Clinical evaluation of the LeukoGuard (LG6) arterial line filter for routine open heart surgery. Perfusion 1993;8:489–496.
20 Allen SM, Pagano D, Bonser RS: Preliminary clinical evaluation of the Pall LeukoGuard (LG6) leukocyte depleting filter. Proc Scand Assoc Thorac Cardiovasc Surg, Tampere, 1993, p 86.
21 Al-Ebrahim K, Shaffei H: Our experience with using the Pall LG6 leukocyte depleting filter during cardiopulmonary bypass. Ann Thorac Surg 1993;56:1216–1217.
22 Palanzo DA, Manley NJ: Cost effectiveness of using a leuko-depleting arterial line filter. Proc Am Acad Cardiovasc Perf 1994;15:124–127.
23 Coleman SM, Demastrie L: Leukocyte depletion reduces postoperative oxygen requirements. Ann Thorac Surg 1994;58:1567–1568.
24 Johnson D, Thompson D, Mycyk T, Burbridge B, Mayers I. Depletion of neutrophils by filtration during aortocoronary bypass surgery transiently improves postoperative cardiorespiratory status. Chest 1995;107:1253–1259.
25 Mihaljevic T, Tonz M, von Segesser LK, et al: The influence of leukocyte filtration during cardiopulmonary bypass on postoperative lung function: A clinical study. J Thorac Cardiovasc Surg 1995;109:1138–1145.
26 Hachida M, Shimabukuro T, Okamura T, et al: The role of leukocyte depletion in reducing injury to the myocardium and lung during cardiopulmonary bypass. ASAIO J 1995;41:291–295.
27 Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W: Leukocyte depletion results in improved lung function and reduced inflammatory response after cardiac surgery. J Thorac Cardiovasc Surg 1996;112:494–500.
28 Bolling KS, Halldorsson A, Allen BS, et al: Prevention of the hypoxic reoxygenation injury with the use of a leukocyte-depleting filter. J Thorac Cardiovasc Surg 1997;113:1081–1090.
29 Di Salvo C, Louca LL, Pattichis K, Hooper J, Walesby RK: Does activated neutrophil depletion on bypass by leukocyte filtration reduce myocardial damage? A preliminary report. J Cardiovasc Surg (Torino) 1996;37(suppl):93–100.
30 Allen BS, Rahman S, Ilbawi MN, et al: Detrimental effects of cardiopulmonary bypass in cyanotic infants: Preventing the reoxygenation injury. Ann Thorac Surg 1997;64:1381–1388.
31 Gott JP, Cooper WA, Schmidt FE Jr, et al: Modifying risk for extracorporeal circulation: Trial of four antiinflammatory strategies. Ann Thorac Surg 1998;66:747–754.
32 Zhang J, Jamieson WR, Sadeghi H, et al: Strategies of myocardial protection for operation in chronic model of cyanotic heart disease. Ann Thorac Surg 1998;66:1507–1513.
33 Hurst T, Johnson D, Cujec B, et al: Depletion of activated neutrophils by a filter during cardiac valve surgery. Can J Anaesth 1997;44:131–139.
34 Sheppard SV, Smith DC, Gibbs RV: Are leucocyte depleting filters capable of selectively removing activated leucocytes during CPB? 25th Anniversary Congress on Perfusion of the Society of Perfusionists in Great Britain and Ireland, Jersey, October 1999.
35 Sawa Y, Matsuda H, Shimazaki Y, et al: Evaluation of leukocyte-depleted terminal blood cardioplegic solution in patients undergoing elective and emergency coronary artery bypass grafting. J Thorac Cardiovasc Surg 1994;108:1125–1131.
36 Pala MG, Paolini G, Paroni R, et al: Myocardial protection with and without leukocyte depletion: A comparative study on the oxidative stress. Eur J Cardiothorac Surg 1995;9:701–706.

37 Ichihara T, Yasuura K, Maseki T, et al: The effects of using a leukocyte removal filter during cold blood cardioplegia. Jpn J Surg 1994;24:966–972.
38 Schmidt FE, MacDonald MJ, Murphy CO, Brown WM 3rd, Gott JP, Guyton RA: Leukocyte depletion of blood cardioplegia attenuates reperfusion injury. Ann Thorac Surg 1996;62:1691–1697.
39 Palatianos GM, Balentine G, Astras G, et al: Leukocyte filtration reduces myocardial reperfusion morbidity. Ann Thorac Surg, in press.
40 Weiss SJ: Tissue destruction by neutrophils. N Eng J Med 1989;320:365–376.
41 Smit JJ, de Vries AJ, Gu YJ, van Oeveren W: Efficiency and safety of leukocyte filtration during cardiopulmonary bypass for cardiac surgery. Transfus Sci 1999;20:151–165.
42 Gu YJ, de Vries AJ, Vos P, Boonstra PW, van Oeveren W: Leukocyte depletion during cardiac operation: A new approach through the venous bypass circuit. Ann Thorac Surg 1999;67:604–609.
43 Allen SM: Leukocyte depletion in cardiothoracic surgery. Perfusion 1996;11:270–277.
44 Baksaas ST, Flom-Halvorsen HI, Ovrum E, et al: Leucocyte filtration during cardiopulmonary reperfusion in coronary artery bypass surgery. Perfusion 1999;14:107–117.
45 Matheis G, Wimmer-Greinecker G, Martens S, Moritz A: Improved organ protection by systemic leukocyte filtration during early reperfusion on CPBG (poster) 19th Annual Pathophysiology and Techniques of Cardiopulmonary Bypass, San Diego, February 1999.
46 Smit JJ, de Vries AJ, Gu YJ, van Oeveren W: Filtration of activated granulocytes during cardiopulmonary bypass surgery: A morphologic and immunologic study to characterize the trapped leukocytes. J Lab Clin Med 2000;135:238–246.

David A. Palanzo, CCP, Perfusion Care Associates, Inc.
P.O. Box 3447, Allentown, PA 18106–0447 (USA)
Tel. +1 610 402 8654, Fax +1 610 402 0013, E-Mail David.Palanzo@LVH.com

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 101–110

Strategic Leukocyte Filtration: Clinical and Experimental Experience

Georg Matheis, Andreas Simon, Martin Scholz

Department of Thoracic and Cardiovascular Surgery, J.W. Goethe University, Frankfurt, Germany

There is broad consensus among investigators, including Gu et al. [1] and Palanzo [2] in this book, that leukocyte filtration may be more efficient in reducing the pathogenicity of cardiopulmonary bypass (CPB) surgery and/or ischemia/reperfusion injury, when leukocyte filters are (1) used to their full capacity, and (2) combined with other technological, pharmaceutical or procedural approaches.

Data obtained from clinical studies carried out during the past decade indicate a beneficial effect of leukocyte filtration applied throughout CPB. In most clinical studies conventional (filtration during the entire perfusion period) leukocyte depletion has been associated with limited release of leukocyte-derived enzymes, improvement of functional outcome (stroke work, cardiac output, myocardial blood flow), and better pulmonary function with significantly improved PaO_2. A major benefit of leukocyte filtration is the significant improvement in pulmonary function [3, 4]. Consequently, improved pulmonary function resulted in significant reduction of time on ventilator, time in the intensive care unit and subsequent cost reduction [5]. However, data presented by Gott et al. [6] in 1998 and Baksaas et al. [7] in 1999 on standard leukocyte filtration, and recent studies performed by Smit et al. [8] on a large-capacity leukocyte filter prototype showed, that efficiency slowed over time. If the leukocyte filter employed throughout CPB is not capable of removing a clinically relevant fraction of (activated) leukocytes at release of the aortic cross clamp, a change of leukocyte filters may be required prior to reperfusion. To date, evidence has accumulated that leukocyte depletion only during the early postischemic reperfusion phase (strategic leukocyte filtration) may be efficient in protecting the heart and lungs [9, 10].

However, strategic leukocyte depletion initiated upon reperfusion does not address other leukocyte-mediated events that occur throughout CPB.

This chapter reports clinical and experimental experience with the so-called 'strategic filtration', which is defined as the focused use of leukocyte filtration (1) during a certain period of time considered most pathogenic, and (2) for a period of time that allows to maintain most of the filter's efficacy. Along with this, the present report integrates important knowledge from previous studies.

Combination of Strategic Leukocyte Filtration with Heparin-Coated CPB Circuits

Numerous studies have assessed heparin-coated or surface-modified extracorporal circuits to reduce the pathogenicity of CPB by limiting inflammatory responses, such as complement and leukocyte activation [11–13]. However, myocardial damage due to reperfusion injury after release of the aortic cross clamp remains largely unaffected by these interventions. Since the pathology of reperfusion injury may be based in part on oxidative stress and leukocyte-mediated disturbance of the endothelial integrity in ischemic tissue, biocompatible linings of the extracorporeal circuit are not expected to significantly reduce reperfusion injury after release of the cross clamp.

Therefore, in a clinical study, we evaluated whether leukocyte filtration in conjunction with heparinized CPB circuits has beneficial effects on myocardial reperfusion injury. In this study leukocyte filters in the arterial line were used only briefly (15 min) during early reperfusion starting with the release of the cross clamp.

Our results provide evidence for the specific benefit of leukocyte filtration on reperfusion-mediated myocardial damage when the CPB circuits were completely heparinized (Bioline coating). Troponin T, a specific marker for myocardial damage during acute myocardial infarction or reperfusion injury [14] was significantly reduced in the filter group 24 h after surgery. In addition, evidence for the beneficial effect of leukocyte filtration was supported by the finding that no patient treated with a leukocyte filter required catecholamines postoperatively, whereas in the control group 3 patients received inotropic support. Experimentally, evidence for the protective effects of leukocyte filtration has been reported by Kraemer and Mullane [15], who found that neutrophils delay the functional recovery of the myocardium in the rabbit, and by Breda et al. [16], who looked at myocardial histology in a porcine model where leukocyte-filtered blood limited subcellular damage versus nonfiltered reperfusate. Overall, these findings indicate that leukocyte filtration alone protects the myocardium

or leads to accelerated myocardial recovery. Our data point to superior effects when heparin-coated circuits are used in combination with strategic leukocyte filtration during CPB [10].

To examine whether strategic leukocyte filtration with heparinized CPB circuits limits aberrant immune responses together with improved myocardial protection, we measured peripheral levels of the proinflammatory cytokine IL-6, the chemokine IL-8 involved in leukocyte activation and attraction. In addition, the end product of the complement cascade, the terminal complement complex C5b9 was analyzed. C5b9 represents an excellent marker for the complement system [17]. In this study, patients who underwent CPB with a heparin-coated system plus leukocyte filtration showed no differences in IL-6 or IL-8 levels, but exhibited significantly reduced plasma levels of C5b9 immediately after filtration and 1 h postoperatively compared to controls treated with heparin coating alone. Furthermore, C5b9 returned to baseline levels even faster than controls. It is important to mention that preoperative levels differed between groups (not shown). Thus, the absolute data should be viewed carefully. Nevertheless, C5b9 declines more rapidly following cross clamp release in the filter group.

It is conceivable that leukocyte depletion limits C5b9 because activated neutrophils responsible for the stimulation of the complement cascade [18, 19] are removed from the circulation. Though it is well documented that heparin-coated circuits alone do reduce C5b9 and granulocyte activation [11–13], we conclude that the combination of these techniques together with increased leukocyte filtration efficacy (strategic filtration) may yield enhanced benefit. This conclusion is supported by the finding that the amount of packed red cells transfused was significantly lower in the treatment group. There were twice as many female patients in the group requiring more transfusions despite lower hematocrit and lower blood volume in women. Di Salvo [20] similarly reported lower amounts of transfusion after leukocyte filtration in the arterial line together with leukocyte filtration of all transfused blood. In addition, postoperative time on ventilator was longer in patients without leukocyte depletion. The latter might be partially due to reduced C5b9 and activated neutrophils, that mediate pulmonary reperfusion injury and cause edema [21–23]. In order to further suppress C5b9, this leukocyte filtration strategy might be used in combination with other means of complement suppression including systemic application of steroids or a specific complement receptor protein called recombinant soluble complement receptor 1 (sCR-1). Contradictory data were presented concerning the use of steroids that are supposed to inhibit C3–C5 convertase [24] or the sCR-1 protein, which was shown to block complement activation in animals [24, 25]. Further studies are necessary to determine the efficacy and cost-benefit ratio of single or combined application of these techniques to reduce inflammatory response to CPB.

From the present data we conclude that the proposed strategic leukocyte depletion strategy reduces myocardial damage during reperfusion following CPB as compared to a heparin-coated system alone. In addition, preliminary evidence for the beneficial effects on systemic inflammatory responses and reduced cerebral injury (reduced neuron-specific enolase levels) was obtained. This finding should be confirmed by further studies in larger cohorts. The combination of heparinized CPB circuits with strategic leukocyte filtration might be utilized for routine CPB management in the future.

Strategic Leukocyte Filtration: Optimal Timing?

The combination of heparinized CPB circuits and strategic leukocyte filtration discussed above led us to investigate the timing of leukocyte filtration in the arterial line. Therefore we randomly assigned patients undergoing routine coronary artery bypass grafting (CABG) to four groups without (group I) or with leukocyte filtration beginning with the onset of CPB (group II), beginning 5 min before aortic declamping (group III) and with aortic declamping (group IV). In this study CPB circuits were not coated with heparin to determine the effects of leukocyte filtration timing alone. Neither strategy seems to be superior to the combination of heparin-coated circuits with short-term leukocyte filtration as reviewed above. Moreover, primary clinical endpoints as well as standard laboratory parameters, leukocyte activation markers or functional neutrophil activity did not clearly differ between groups. For example elastase, myeloperoxidase (MPO) or MDA increased to similar amounts during CPB and/or reperfusion, regardless of the filtration strategy. To assess the impact of the filters on leukocyte count and activity, blood was collected immediately before and after passing the leukocyte filter (LG6, Pall). The total leukocyte count increased similarly in all groups and no gradient in the percentage of the neutrophil population before and after filtration could be found. Furthermore, neutrophil CD18 expression as well as the functional parameters phagocytosis (labeled *Escherichia coli*) and oxidative burst were measured by flow cytometry. In patients without leukocyte filtration the expression of CD18 increased during CPB and reperfusion, and baseline levels of CD18 expression declined and remained low after onset of CPB and activation of the filter, indicating that the filter depletes activated rather than inactivated neutrophils. Considering the rapid release of neutrophils from bone marrow and the demargination of neutrophils, the absolute number of leukocytes might not be affected in the first line. Therefore, it is necessary to determine the influence of leukocyte filtration on the functional activity of neutrophils. The ability of neutrophils to phagocytose bacteria (labeled *E. coli*), and to elicit an oxidative burst after stimulation

with *E. coli* can be measured by flow cytometry. This is an important tool to evaluate functional activity. Phagocytotic activity of neutrophils isolated from blood collected immediately behind the filter seemed not to be reduced by filtration. Similar results were found for the oxidative burst. However, in some cases the extent of oxidative burst stimulated in vitro was rather pronounced in neutrophil populations that had passed the filter compared with those collected before the passage. It can be speculated that neutrophils which pass the filter may have retained their capacity to elicit oxidative burst, and thus still contribute to oxidative damage despite leukocyte filtration.

In another clinical study, the efficacy of leukocyte filtration was evaluated in high-risk patients. Leukocyte filters in the arterial line were activated 10 min before release of the cross clamp until the end of CPB. There was no demographic difference between patient groups. However, as reported by Mair et al. [26], elastase levels were significantly enhanced after release of the cross clamp as determined by ELISA (quantification of the inactive complex).

Similar to the clinical study with heparinized CPB circuits in combination with strategic filtration, troponin T was significantly reduced in the filter group. This finding further confirms the suggestion that leukocyte filtration before/during reperfusion has protective effects on the myocardium. Moreover, we observed significantly lower leukocyte counts in the filter group at 24 h after surgery, whereas during and early after operation no significant difference between groups was found. The latter results are intriguing since leukocyte filtration might have additional clinical benefits via an indirect influence on neutrophil release from bone marrow, which plays a crucial role in the development of SIRS.

In conclusion, these results advocate leukocyte depletion strategies beyond current technology. In the following paragraphs, novel efforts to deplete leukocytes in cardiac surgery with CPB are proposed. These concepts may further limit CPB-associated pathogenicity, when combined with strategic filtration.

Leukocyte Depletion in the Venous Line

The recent study by Gu et al. [27] showed the feasibility of low-flow filtration in the venous line. In this study leukocyte filtration was found to reduce neutrophil counts and the potent chemoattractant IL-8. A more recent study supported the feasibility of this procedural modification [28]. The authors showed significant removal of activated leukocytes, and further suggested the absence of activation of granulocytes by filtration because β-glucuronidase concentration did not increase after filtration.

Leukocyte Filtration of Blood Cardioplegia

Despite numerous modifications of CPB including heparin coating [11, 13] protein coating [12], surface modification [29], or pulsatile CPB [30], myocardial damage due to reperfusion injury after release of the aortic cross clamp remains largely unaffected by these interventions, and has been advanced mainly by the introduction of blood cardioplegic regimens [31]. The pathology of reperfusion injury might be largely based on oxidative stress and leukocyte-mediated disturbance of endothelial integrity. There is considerable body of evidence regarding selective myocardial protection by leukocyte filters in the blood cardioplegia line [32–34]. The repeated reperfusion of the coronary bed with each reinfusion of cardioplegia is a highly pathogenic situation. The coronary circulation cannot be addressed by arterial inline leukocyte filters during aortic cross-clamping unless the blood used for cardioplegia is drawn behind the arterial filter. A specific leukocyte-depleting blood cardioplegia filter (BC1, Pall) has been developed and shown to be safe and efficient in terms of low resistance, leukocyte reduction (70%), low platelet depletion rate [35]. It has been demonstrated that leukocyte depletion of blood cardioplegia reduces myocardial injury and improves cardiac function, as determined by stroke work index, limits necrosis, and endothelial dysfunction with edema. More specifically, Sawa et al. [36] found reduced creatine kinase-MB (CK-MB) concentrations, reduced oxidative damage and reduced inotropic support. Similarly, by using cold blood cardioplegia, Ichihara et al. [37] have noted lower values of lipid peroxide, elastase and CK-MB in the leukocyte-depleted group. Recently, Hayashi et al. [38] and Roth et al. [39] have confirmed that myocardial cell injury can be significantly affected by the use of leukocyte-depleted blood cardioplegia as a result of limited oxidative damage (e.g. lower MDA levels in coronary sinus blood).

Leukocyte Filtration of Cardiotomy Suction Blood

Leukocyte filtration of cardiotomy suction and salvaged chest drain blood is an another important issue that should be addressed. Untreated suction blood contains significant amounts of tissue debris, soluble factors such as proinflammatory cytokines and activated leukocytes. Gu et al. [40] investigated residual blood from a heart-lung machine, and found that leukocyte depletion of this blood improves postoperative pulmonary gas exchange function. However, recent studies [41] indicate that the pathogenicity of cardiotomy suction blood be prevented more efficiently by abolishing the reperfusion of cardiotomy suction blood in cases with lower suction volume such as routine CABG. The experience with reservoirless CPB confirms this evidence. In the remaining

cases where cardiotomy suction cannot be avoided leukocyte filtration may be used in conjunction with strategies to avoid the retransfusion of lipids that have been found to accumulate in the cerebral circulation [41].

General Considerations

Although there is a large body of evidence on the beneficial effects of leukocyte filtration in cardiac surgery, controversial results have been reported by several authors. In an in vitro study, no significant intergroup difference was found between extracorporeal circuits equipped with a standard filter versus a leukocyte filter [42]. Thurlow et al. compared the efficacy of a standard filter with the Pall LG6 (Leukoguard) and did not find any significant difference in the phenotypic expression of activation markers on filtered neutrophils, except lower tyrosine phosphate CD45Ro in the leukocyte filter group. These authors report on the reduction of neutrophils that produce superoxide as determined by dihydrorhodamine-123 when the leukocyte filter was used. They conclude that the neutrophil filter is not capable of significantly depleting the neutrophil load generated during CPB, but may selectively remove the more activated forms [43]. Mair et al. [26] showed increased plasma elastase levels in the filter group (as mentioned above), but failed to identify significant differences in PaO_2, C-reactive protein, CK-MB, and troponin I between groups (Pall-LG-6 versus AV-6) in 40 patients undergoing coronary artery bypass surgery. Similar results were obtained by Mihaljevic et al. [44]. A study conducted by Al-Ebrahim et al. [45] showed that leukocyte depletion had no effect on time on ventilator or peripheral white cell count. In a retrospective study in infants undergoing correction of congenital heart defects no difference in outcome was observed between the leukocyte filter group (LG-3; $n = 6$) and controls ($n = 6$) [46]. Despite effective depletion of activated leukocytes, as determined by leukocyte CD18 expression, no clinical benefit or effect on immunological parameters, such as IL-6 and IL-8, was found in valve surgery with leukocyte-depleting filters [47]. It seems difficult to compare the results of these studies because the complex management of CPB, and surgical techniques vary widely. However, it is apparent that the duration of CPB may influence the efficacy of leukocyte depletion. In this regard, leukocyte filtration positively (improved pulmonary outcome) correlated with the duration of CPB after aortic cross-clamping and cardioplegic arrest for 90 min [21]. Blood temperature seems to influence the efficacy of leukocyte depletion as well. Mild hypothermia during CPB resulted in further reduced leukocyte counts.

We believe that strategic leukocyte filtration yields substantial benefits and should be combined with other proven leukocyte filtration concepts (transfusion,

blood cardioplegia, cardiotomy suction). In a multifaceted clinical situation such as cardiac surgery with CPB a multifactorial approach may be wise that combines leukocyte filtration with other approaches to reducing the pathogenicity of CPB such as reservoirless CPB where possible (i.e. routine CABG).

References

1 Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W: Mechanism and strategy of leukocyte depletion during cardiopulmonary bypass surgery; in Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology. Basel, Karger, 2002, pp 78–88.

2 Palanzo DA: Leukocyte filters in perfusion; in Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology. Basel, Karger, 2002, pp 89–100.

3 Johnson D, Thompson D, Mycyk T, Burbridge B, Mayers I: Depletion of neutrophils by filter during aortocoronary bypass surgery transiently improves postoperative cardiorespiratory status. Chest 1995;107:1253–1259.

4 Ohto T, Yamamoto F, Nakajima N: Evaluation of leukocyte-reducing arterial line filter (LG6) for postoperative lung function, using cardiopulmonary bypass. Jpn J Thorac Cardiovasc Surg 2000; 48:295–300.

5 Palanzo DA, Manley NJ, Montesano RM, Yeisley GL, Gordon D: Clinical evaluation of the LeukoGuard (LG6) arterial line filter for routine open heart surgery. Perfusion 1993;8:489–496.

6 Gott JP, Cooper WA, Schmidt FE Jr, Brown WM 3rd, Wright CE, Merlino JD, Fortenberry JD, Clark WS, Guyton RA: Modifying risk for extracorporeal circulation: Trial of four antiinflammatory strategies. Ann Thorac Surg 1998;66:747–753.

7 Baksaas ST, Flom-Halvorsen HI, Ovrum E, Videm V, Mollnes TE, Brosstad F, Svennevig JL: Leucocyte filtration during cardiopulmonary reperfusion in coronary artery bypass surgery. Perfusion 1999;14:107–117.

8 Smit JJ, de Vries AJ, Gu YJ, van Oeveren W: Efficiency and safety of leukocyte filtration during cardiopulmonary bypass for cardiac surgery. Transfus Sci 1999;20:151–165.

9 Levine AJ, Parkes K, Rooney S, Bonser RS: Reduction of endothelial injury after hypothermic lung preservation by initial leukocyte-depleted reperfusion. J Thorac Cardiovasc Surg 2000;120: 47–54.

10 Matheis G, Scholz M, Gerber J, Abdel-Rahman U, Wimmer-Greinecker G, Moritz A: Leukocyte filtration in the early reperfusion phase on cardiopulmonary bypass reduces myocardial injury. Perfusion 2001;16:43–49.

11 Moen O, Hagasen K, Fosse E, et al: Attenuation of changes in leukocyte surface markers and complement activation with heparin-coated cardiopulmonary bypass. Ann Thorac Surg 1997;63: 105–111.

12 Fosse E, Thelin S, Svennevig JL, et al: Duraflo-II-coating of cardiopulmonary bypass circuits reduces complement activation, but does not affect the release of granulocyte enzymes: A European multicenter study. Eur J Cardiothorac Surg 1997; 11:320–327.

13 Ovrum E, Fosse E, Mollnes TE, et al: Complete heparin-coated cardiopulmonary bypass and low heparin dose reduce complement and granulocyte activation. Eur J Cardiothorac Surg 1996; 10:54–60.

14 Mair J: Cardiac troponin I and troponin T: Are enzymes still relevant as cardiac markers? Clin Chim Acta 1997;257:99–115.

15 Kraemer R, Mullane KM: Neutrophils delay functional recovery of the post-hypoxic heart of the rabbit. J Pharmacol Exp Ther 1989;251:620–626.

16 Breda MA, Drinkwater DC, Laks H, et al: Prevention of reperfusion injury in the neonatal heart with leukocyte depleted blood. J Thorac Cardiovasc Surg 1989;97:654–665.

17 Videm V, Fosse E, Mollnes TE, Garred P, Svennevig JL: Time for new concepts about measurement of complement activation by cardiopulmonary bypass. Ann Thorac Surg 1992;54:725–731.

18 Butler J, Rocker GM, Westaby S: Inflammatory response to cardiopulmonary bypass. Ann Thor Surg 1993;55:552–559.
19 Engler R: Consequences of activation and adenosine-mediated inhibition of granulocytes during myocardial ischemia. Fed Proc 1987;46:2407–2412.
20 Di Salvo C: Does activated neutrophil depletion on bypass by leukocyte filtration reduce myocardial damage? A preliminary report. J Cardiovasc Surg (Torino) 1996;37(suppl):93–100.
21 Bando K, Pillai R, Cameron DE, et al: Leukocyte depletion ameliorates free radical-mediated lung injury after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;99:873–877.
22 Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W: Leukocyte depletion results in improved lung function and reduced inflammatory response after cardiac surgery. J Thorac Cardiovasc Surg 1996;112:494–500.
23 Grace PA: Ischemia-reperfusion injury. Br J Surg 1994;81:637–647.
24 Moat NE, Shore D, Fevans TW: Organ dysfunction and cardiopulmonary bypass: The role of complement and complement regulatory proteins. Eur J Cardiothorac Surg. 1993;7:563–573.
25 Weisman HF, Bartow T, Leppo MK, et al: Soluble human receptor type 1: In vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. Science 1990;249: 146–151.
26 Mair P, Hoermann C, Mair J, Margreiter J, Puschendorf B, Balogh D: Effects of a leucocyte depleting arterial line filter on perioperative proteolytic enzyme and oxygen free radical release in patients undergoing aortocoronary bypass surgery. Acta Anaesthesiol Scand 1999;43:452–457.
27 Gu YJ, de Vries AJ, Vos P, Boonstra PW, van Oeveren W: Leukocyte depletion during cardiac operation: A new approach through the venous bypass circuit. Ann Thorac Surg 1999;67:604–609.
28 Smit JJ, de Vries AJ, Gu YJ, van Oeveren W: Filtration of activated granulocytes during cardiopulmonary bypass surgery: A morphologic and immunologic study to characterize the trapped leukocytes. J Lab Clin Med 2000;135:238–246.
29 Gu YJ, Boonstra PW, Rijnsburger AA, Haan J, van Oeveren W: Cardiopulmonary bypass circuit treated with surface-modifying additives: A clinical evaluation of blood compatibility. Ann Thorac Surg 1998;65:1342–7134.
30 Taylor KM: Pulsatile reperfusion: The effects on coronary blood flow and myocardial ischemic injury. J Thorac Cardiovasc Surg 1983;85:941–942.
31 Buckberg GD: Oxygenated cardioplegia: Blood is a many splendored thing. Ann Thorac Surg 1990;50:175–177.
32 Suzuki I, Ogoshi N, Chiba M, Komatsu T, Moizumi Y: Clinical evaluation of a leucocyte-depleting blood cardioplegia filter (BC1B) for elective open-heart surgery. Perfusion 1998;13:205–210.
33 Kofsky ER, Julia PL, Buckberg GD, Quillen JE, Acar C: Studies of controlled reperfusion after ischemia. XXII. Reperfusate composition: Effects of leukocyte depletion of blood and blood cardioplegic reperfusates after acute coronary occlusion. J Thorac Cardiovasc Surg 1991;101: 350–359.
34 Schmidt FE Jr, MacDonald MJ, Murphy CO, Brown WM 3rd, Gott JP, Guyton RA: Leukocyte depletion of blood cardioplegia attenuates reperfusion injury. Ann Thorac Surg 1996;62: 1691–1696.
35 Heggie AJ, Corder JS, Crichton PR, Hesford JW, Bingham H, Jeffries S, Hooper TL: Clinical evaluation of the new Pall leucocyte-depleting blood cardioplegia filter (BC1). Perfusion 1998;13:17–25.
36 Sawa Y, Schaper J, Roth M, Nagasawa K, Ballagi G, Bleese N, Schaper W: Platelet-activating factor plays an important role in reperfusion injury in myocardium. Efficacy of platelet-activating factor receptor antagonist (CV-3988) as compared with leukocyte-depleted reperfusion. J Thorac Cardiovasc Surg 1994;108:953–959.
37 Ichihara T, Yasuura K, Maseki T, Matsuura A, Miyahara K, Ito T, Kato S, Mizuno S, Tamaki S, Seki A, et al: The effects of using a leukocyte removal filter during cold blood cardioplegia. Surg Today 1994;24:966–972.
38 Hayashi Y, Sawa Y, Nishimura M, Ichikawa H, Kagisaki K, Ohtake S, Matsuda H: Clinical evaluation of leukocyte-depleted blood cardioplegia for pediatric open heart operation. Ann Thorac Surg 2000;69:1914–1919.

39 Roth M, Kraus B, Scheffold T, Reuthebuch O, Klovekorn WP, Bauer EP: The effect of leukocyte-depleted blood cardioplegia in patients with severe left ventricular dysfunction: A randomized, double-blind study. J Thorac Cardiovasc Surg 2000;120:642–650.
40 Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W: Clinical performance of a high efficacy rapid flow leukocyte removal filter for leukocyte depletion of heparinized cardiopulmonary bypass perfusate. Perfusion 1995;10:425–430.
41 Brooker RF, Brown WR, Moody DM, Hammon JW, Reboussin DM, Deal DD, Ghazi-Birry HS, Stump DA: Cardiotomy suction: A major source of brain lipid emboli during cardiopulmonary bypass. Ann Thorac Surg 1998;65:1651–1665.
42 Baksaas ST, Videm V, Mollnes TE, Pedersen T, Karlsen H, Svennevig JL: Effects on complement, granulocytes and platelets of a leukocyte-depletion filter during in vitro extracorporeal circulation. Scand Cardiovasc J 1997;31:73–77.
43 Thurlow PJ, Doolan L, Sharp R, Sullivan M, Smith B, Andersen LW: Laboratory studies of the effect of Pall extracorporeal leucocyte filters LG6 and AV6 on patients undergoing coronary bypass grafts. Perfusion 1996;11:29–37.
44 Mihaljevic T, Tonz M, von Segesser LK, et al: The influence of leukocyte filtration during cardiopulmonary bypass on postoperative lung function: A clinical study. J Thorac Cardiovasc Surg 1995;109:1138–1145.
45 Al-Ebrahim K, Shafei H: Our experience with using the Pall LG6 leukocyte depleting filter during cardiopulmonary bypass. Ann Thorac Surg 1993;56:1216–1217.
46 Englander R, Cardarelli MG: Efficacy of leukocyte filters in the bypass circuit for infants undergoing cardiac operations. Ann Thorac Surg 1995;60:S533–S535.
47 Hurst T, Johnson D, Cujec B, Thomson D, Mycyk T, Burbridge B, Mayers I: Depletion of activated neutrophils by a filter during cardiac valve surgery. Can J Anaesth 1997;44:131–139.

Dr. G. Matheis, Jostra Medizintechnik AG, D–72145 Hirrlingen (Germany)
Tel. +49 7478 921 418, Fax +49 7478 921 400, E-Mail georg.matheis@jostra.net

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 111–134

Hypoxia, Reoxygenation, and the Role of Leukodepletion in the Intraoperative Management of Congenital Heart Disease

Bradley S. Allen

Division of Cardiovascular Surgery, Heart Institute for Children,
Hope Children's Hospital, Oak Lawn, Ill., USA

Repair of cyanotic congenital heart defects necessitating cardiopulmonary bypass (CPB) is becoming more frequent in infants and neonates. Despite apparently successful surgical correction, postoperative myocardial and pulmonary dysfunction continues to be a major contributor to morbidity and mortality, and is more severe than after repair of acquired defects in adults with normoxic conditions [1–3]. The neonatal heart also has a reduced response to inotropic agents compared to the adult [2–4]. Thus, preservation of myocardial function in neonates during cardiac operations assumes even greater importance, because a perioperative insult is less well tolerated and more difficult to treat.

Compared to adults, pediatric patients are normally subjected to different preoperative myocardial stresses, and so the concerns during CPB are not necessarily the same. The most common preoperative stress in adults is ischemia, secondary to coronary artery disease, and the major concern is avoidance of a reperfusion injury with the reintroduction of blood [5]. In contrast, the most common preoperative stress in pediatric patients is hypoxia (cyanosis) [2, 3]. The major concern, therefore, is whether damage (similar to a reperfusion injury) occurs with the abrupt reintroduction of oxygen. The occurrence of such an injury could be even more detrimental, since hypoxia affects all organs, not just the heart.

The normoxic immature myocardium has increased tolerance to ischemia because of adaptive mechanisms, which has led to the perception pediatric hearts are less likely to undergo an injury with bypass or surgical ischemia [2, 3, 6].

However, recent reports have shown that cyanotic (hypoxic) hearts are less tolerant to intraoperative ischemia than normoxic hearts [3, 7–9]. Chronic cyanosis depletes the myocardium of endogenous antioxidants, and a growing body of experimental and clinical evidence indicates that this may make the cyanotic immature heart more susceptible to an oxygen-mediated injury when molecular oxygen is restored [10–15]. The reversal of hypoxemia occurs with initiation of extracorporeal circulation and precedes the surgical ischemia used for operative repair in children with cyanotic disease. The conventional method of starting CPB in infants and children with hypoxemia is to abruptly raise oxygen tension (PO_2) to approximately 400–500 mm Hg. This sudden reintroduction of oxygen may cause an 'unintended injury', which would explain why the cyanotic heart is less tolerant to surgical ischemia.

Oxygen free radicals are one of the principal mediators of the reperfusion injury, and they may also be a central effector of a reoxygenation injury, since both hypoxia and ischemia expose tissue to low levels of oxygen, and CPB generates reactive oxygen intermediates by altering neutrophils [10, 11, 16–21]. Reoxygenation affects tissue other than the myocardium, further complicating the postoperative course of a patient with altered cardiac function. For example, studies of lung reoxygenation show that a pulmonary injury follows the initiation of extracorporeal membrane oxygenation (ECMO) for respiratory failure or re-expansion after chronic atelectasis [22, 23]. Additionally, vascular endothelium is a well-recognized target with reintroduction of molecular oxygen, because reperfusion reduces coronary endothelial nitric oxide (NO) production and subsequent endothelial dependent relaxation, and potentiates endothelial-derived contraction to favor coronary vasospasm [24–26]. A similar change may occur in the lung so that the impaired capacity to produce endogenous NO favors pulmonary vasoconstriction, and exogenous NO delivery may be required to reduce pulmonary vascular resistance [27]. Reoxygenated endothelial cells release superoxide anion, causing damage and alteration of barrier function. Endothelial cell membrane injury also promotes subsequent neutrophil adherence and activation, leading to capillary plugging, reduced flow and further release of oxidants.

This chapter details our experimental and subsequent clinical experience with the injury caused by abrupt reoxygenation of the hypoxic (cyanotic) heart. It then examines the modalities of gradual reoxygenation, leukodepletion and cardioplegia in limiting and repairing this injury, thereby improving operative outcomes for cyanotic lesions. This will lead to the conclusion that (1) the reoxygenation injury is a real source of postoperative cardiac and pulmonary dysfunction, (2) white blood cells play an integral role in the production of oxygen free radicals which are responsible for the damage, and (3) this injury can be modified and possibly ameliorated by changes in intraoperative management during CPB in children with cyanotic disease.

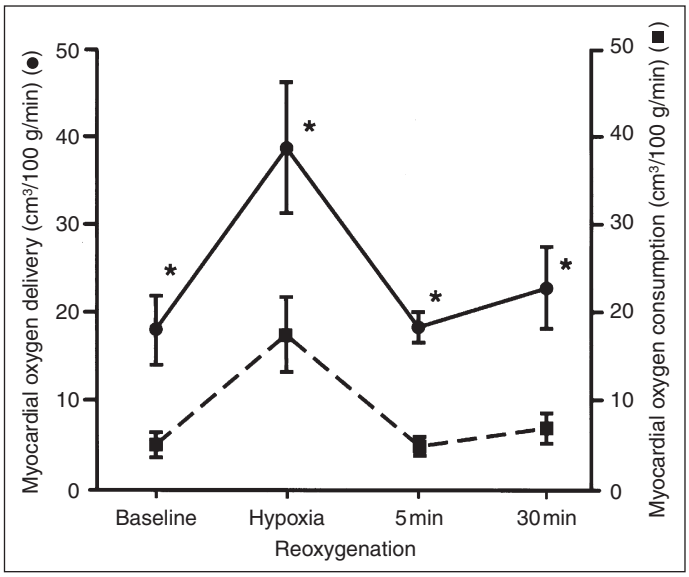


Fig. 1. Myocardial oxygen consumption and delivery at baseline, at the end of 60 min of hypoxia, and 5 and 30 min following abrupt reoxygenation with 100% oxygen. *$p < 0.05$ vs. oxygen consumption.

Experimental Studies

We initially examined the consequences of hypoxia and reoxygenation using an in vivo piglet model to simulate the cyanotic infant undergoing surgical repair [28]. Neonatal piglets underwent 60 min of ventilator hypoxia by lowering the fraction of inspired oxygen to 8–10%, producing an arterial oxygen tension of 25–35 mm Hg, and an oxygen saturation of 65–70%. Before hypoxemia, piglets were transfused as necessary to increase the hematocrit value to greater than 35%. This simulates the chronic adaptive change of erythrocytosis that occurs in the cyanotic infant and increases oxygen carrying capacity. Myocardial oxygen delivery was further increased during hypoxia by coronary vasodilatation, resulting in an oxygen delivery that exceeded demands (fig. 1, 2). During hypoxia, there was no lactate production or acidosis across the myocardium, ATP levels were preserved, there was no change in cardiac output, and all animals remained hemodynamically stable. Therefore, this was a pure hypoxic stress, without any evidence of ischemia. Animals were then abruptly reoxygenated by increasing the ventilator fraction of inspired oxygen (FiO_2) to 100%, or placed on CPB at an FiO_2 of 100%, simulating the usual clinical practice. Abrupt reoxygenation by either method caused an oxygen-free-radical-mediated

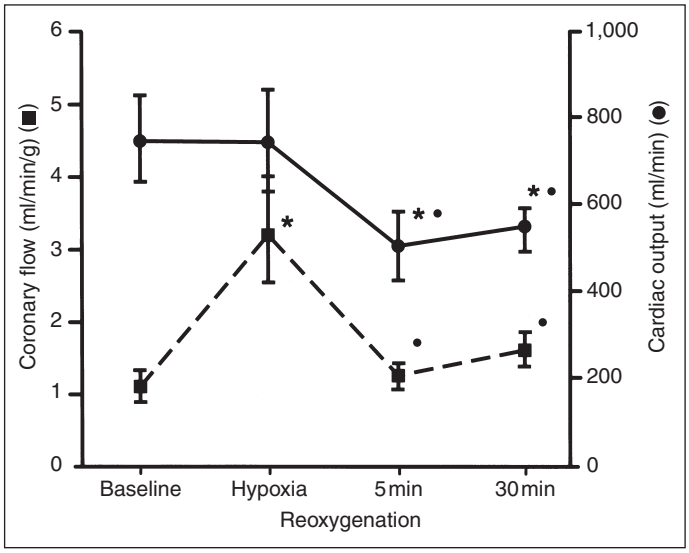


Fig. 2. Cardiac output and coronary flow at baseline, at the end of 60 min of hypoxia, and 5 and 30 min following abrupt reoxygenation with 100% oxygen. *$p < 0.05$ vs. baseline, •$p > 0.05$ vs. hypoxia.

injury, documented by an increase in conjugated dienes and a loss of myocardial antioxidant reserve capacity (fig. 3). The antioxidant reserve capacity is determined by adding a strong oxidant (t-butyl hydroperoxide) to myocardial tissue and measures the tissues' ability to scavenge the resulting oxygen radicals, and prevent malondialdehyde (MDA) production, which is a byproduct of lipid peroxidation [28]. Therefore, it tests the endogenous stores of oxygen radical scavengers. A loss of tissue antioxidants occurs when oxygen free radicals are produced and need to be scavenged, such as when the hypoxemic heart undergoes abrupt reoxygenation using CPB. Increased production of oxygen free radical then caused a reduction in cardiac output, depressed LV function, elevated pulmonary vascular resistance, and pulmonary alveolar damage manifested by a reduction in the arterial/alveolar (a/A) ratio (fig. 2, 4). This injury was independent of the method of reoxygenation, and so it appears to be primarily related to the sudden reintroduction of oxygen, and not to the effects of CPB. This unintended injury could explain why cyanotic infants are more sensitive to surgical ischemia, and often experience myocardial dysfunction despite performing an apparently technically successful operation with '*good*' myocardial protection [2, 3, 7–11, 29].

Since the cyanotic (hypoxic) neonatal myocardium is susceptible to a reoxygenation injury during the abrupt reintroduction of molecular oxygen, and

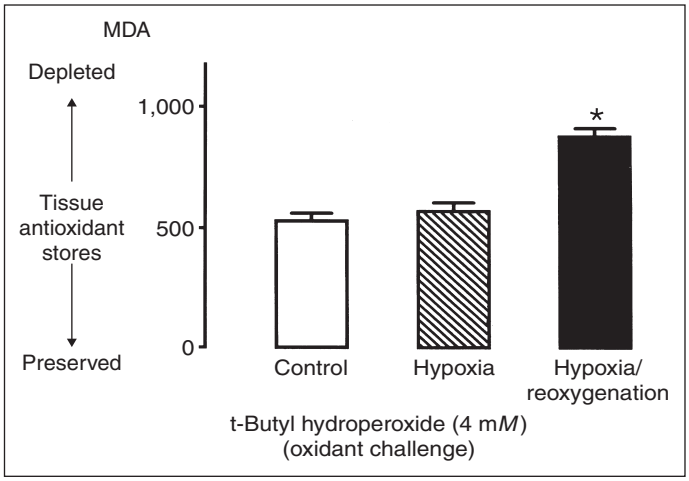


Fig. 3. Myocardial tissue antioxidant reserve capacity at baseline (control), at the end of hypoxia, and after the abrupt reintroduction of oxygen (reoxygenation, 100% FiO_2), either by increasing the oxygen in the ventilator, or initiating CPB. The more MDA (nmol/g protein) produced by an oxidant challenge (4 m*M* t-butyl hydroperoxide), the greater the loss of endogenous tissue antioxidants, indicating exposure to increased levels of oxygen free radicals during reoxygenation. *$p < 0.05$.

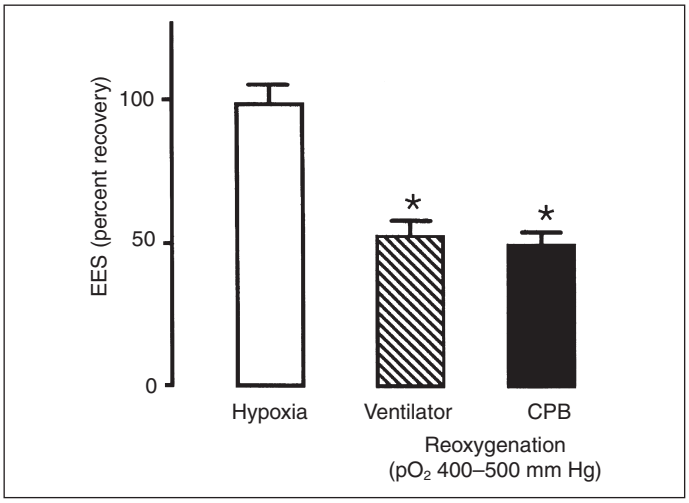


Fig. 4. Percent recovery of end systolic elastance (EES) compared to baseline values, at the end of hypoxia, and after the abrupt reintroduction of oxygen (reoxygenation, 100% FiO_2), either by increasing the oxygen in the ventilator, or initiating CPB. *$p < 0.05$ vs. hypoxia.

this damage is produced to oxygen free radicals, it may be possible to minimize the reoxygenation injury by either gradually restoring oxygen levels, or leukodepleting the blood, as white blood cells are a primary source of oxygen free radicals [20, 21, 30–32]. We tested both of these hypotheses in our acute hypoxic piglet model [28]. Instead of abrupt reoxygenation using a PO_2 400–500 mm Hg (FiO_2 100%), CPB was initiated in one group of piglets at an arterial oxygen saturation of 80–100 mm Hg and the fraction of inspired oxygen gradually increased to 50% over 10–20 min. In another group the bypass blood prime was completely leukodepleted (Pall RC-400) and an inline arterial filter (Pall BC-1) inserted for continual removal of white blood cells.

Maintaining normoxemia (PO_2 80–100 mm Hg) rather than hyperoxemia substantially reduced oxidant damage (lipid peroxidation and depletion of antioxidant reserve capacity) and decreased the extent of myocardial dysfunction (fig. 5, 6). These benefits coincide with the PO_2-dependent nature of the reoxygenation injury, because free radical production and myocardial injury after reoxygenation of isolated heart preparations are proportionate to oxygen tension [33, 34]. In clinical practice hyperoxemic bypass is performed routinely but is likely never needed, because a $PO_2 > 100$–150 mm Hg confers an only negligible increase in O_2 content, and has been associated with an impairment in peripheral perfusion [35]. The avoidance of hyperoxemia during reoxygenation in cyanotic infants to reduce injury may in a sense be comparable with controlling the initial reperfusate following ischemia to avoid a reperfusion injury [5].

Although white blood cells (WBCs) are involved mainly in the maintenance of the immune system, under certain pathologic conditions of altered physiology they may cause damage to myocardial, pulmonary, or vascular tissue [20, 21, 30, 32]. Activated white cells have been shown to play a major role in the generation of oxygen free radicals after ischemia [20, 21, 36, 37]. Therefore, it seems likely that they are also active in the reoxygenation injury since both ischemia and hypoxia subject tissue to low levels of oxygen. Leukocyte depletion is a readily available method which allows the surgeon to safely minimize the harmful effects of neutrophils, without risking side effects of pharmacologic interventions aimed at altering leukocyte function, or preventing the free radical injury through the use of exogenous oxygen radical scavengers. WBCs were effectively reduced by filtration during the entire period of bypass (fig. 7). Activated WBCs (which cause the damage) also bind to activated platelets, and since activated WBC/platelet complexes are larger, they should more likely be trapped by a filter. Therefore, even though the neutrophil count was not reduced to 0, it is probable that very few activated WBCs escaped filtration. In addition, by removing activated platelets a WBC filter may help prevent the adverse effects of thromboxane release and vasoconstriction [20, 32, 36].

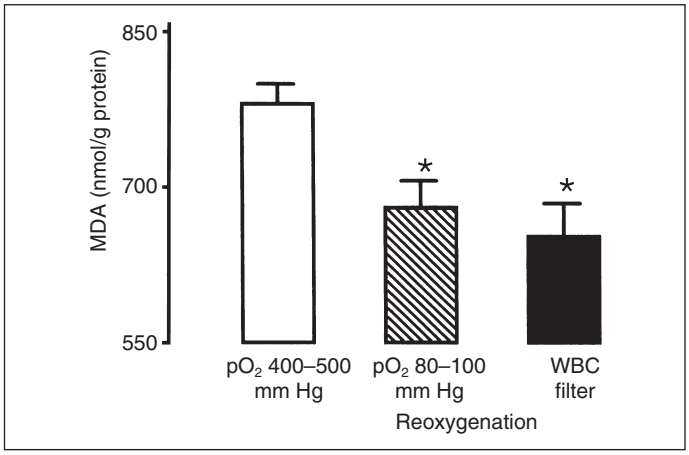


Fig. 5. Myocardial tissue antioxidant reserve capacity in animals undergoing CPB after abrupt reoxygenation at a pO_2 of 400–500 mm Hg, gradual reoxygenation at a pO_2 of 80–100 mm Hg, or leukodepletion. *$p < 0.05$ vs. pO_2 400–500 mm Hg.

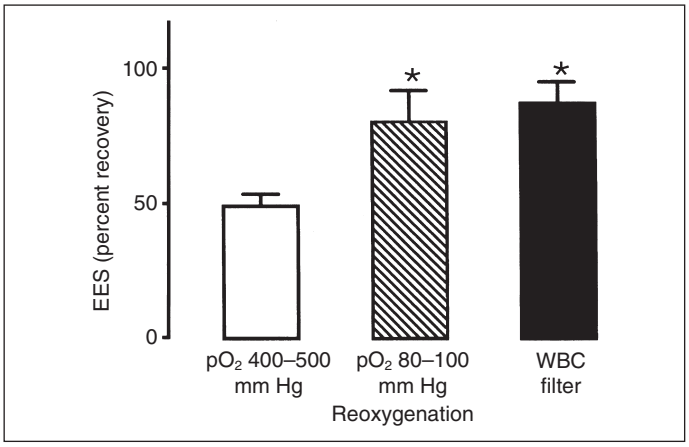


Fig. 6. Percent recovery of end systolic elastance (EES) compared to baseline values in animals undergoing CPB, after abrupt reoxygenation at a pO_2 of 400–500 mm Hg, gradual reoxygenation at a pO_2 of 80–100 mm Hg, or leukodepletion. *$p < 0.05$ vs. pO_2 400–500 mm Hg.

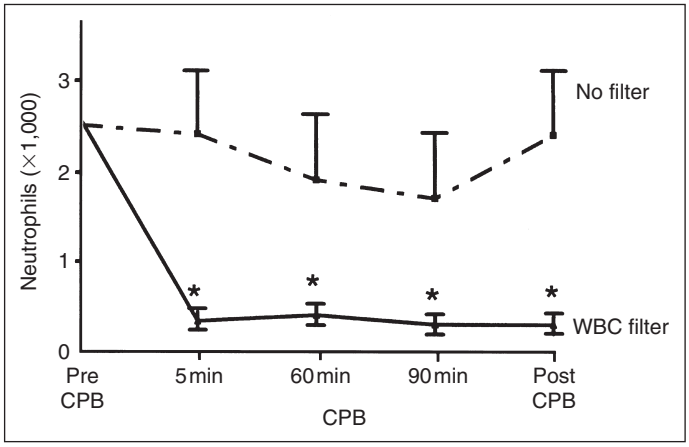


Fig. 7. Neutrophil count in animals undergoing CPB with and without a WBC filter. *$p < 0.05$.

When neutrophils were reduced by a leukocyte-depleting filter, the detrimental effects of sudden reoxygenation were obviated, with a marked reduction in oxygen free radical formation, preservation of LV contractility and diastolic compliance, maintenance of pulmonary alveolar capillary gas exchange (a/A ratio), and only a slight rise in pulmonary vascular resistance (fig. 5, 6, 8, 9). This occurred despite using an FiO_2 of 100% in the leukodepleted group. In fact, the pulmonary vascular resistance in these piglets was even less than in nonhypoxic (control) animals subjected to CPB, suggesting that leukocyte filtration should be used in all pediatric operations where postoperative pulmonary hypertension could be problematic. Several experimental and clinical studies support this implication, and in fact have documented a reduction in pulmonary injury with leukofiltration in noncyanotic infants [17, 38].

Neutrophils have a variety of deleterious effects [20, 21, 30, 36]. Under conditions of hypoxemia or ischemia, coronary vascular endothelium expresses sites which bind neutrophils on reperfusion [20, 26, 32, 36]. Once bound, the neutrophil may be activated by several different pathways including superoxide production by xanthine oxidase, complement activation, and leukotriene production. The bound activated neutrophil is then involved in pathways which contribute to myocyte injury, as well as releasing substances which are chemotatic for other neutrophils and macrophages, resulting in an amplification of the inflammatory response [30, 39]. Although WBCs can injure tissue by several mechanisms, numerous studies have demonstrated that production of oxygen

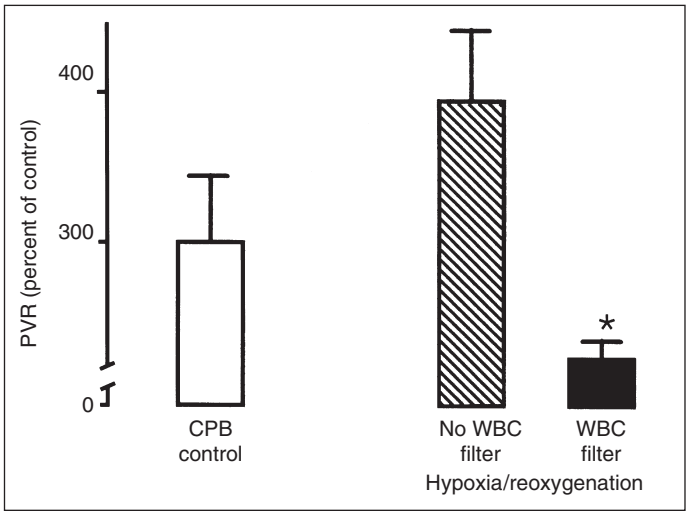


Fig. 8. Pulmonary vascular resistance (PVR) compared to baseline in animals undergoing CPB, without hypoxia (controls), and in hypoxic animals after abrupt reoxygenation at a pO_2 of 400–500 mm Hg with or without a WBC filter. *$p < 0.05$ vs. other groups.

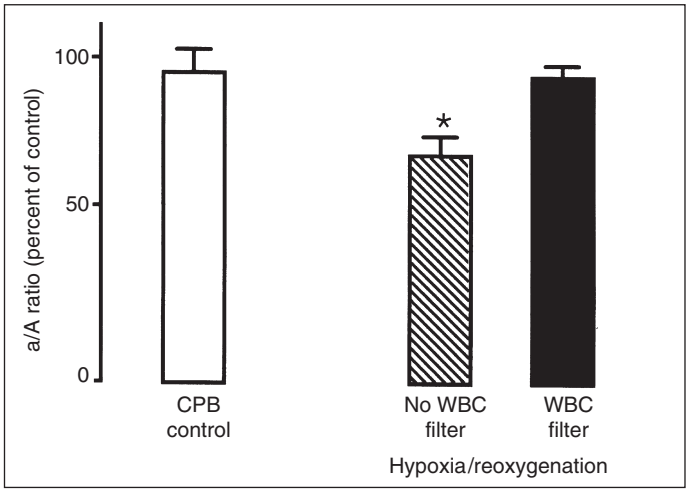


Fig. 9. Recovery of arterial/alveolar (a/A) ratio compared to baseline in animals undergoing CPB, without hypoxia (controls), and in hypoxic animals after abrupt reoxygenation at a pO_2 of 400–500 mm Hg, with or without a WBC filter. *$p < 0.05$ vs. other groups.

radicals is probably the major factor responsible for cellular damage [19–21, 31, 32, 39]. The preservation of antioxidant reserve capacity with leukodepletion (fig. 5) supports this hypothesis, and suggests that (1) activated neutrophils are a major source of the oxygen free radicals produced as a consequence of reoxygenation, and (2) since postbypass ventricular and pulmonary function directly correlate with oxygen reserve capacity, confirms that a major component of the reoxygenation injury responsible for organ dysfunction is oxygen free radicals.

Critique of Experimental Model

The advantages of the intact animal model have been enumerated previously [40]. Five-to-eighteen-day-old piglets approximate the physical conditions of a human neonate, but differences between it and the newborn infant must still be evaluated. An important shortcoming of the short-term or acute hypoxic model is the absence of the ability to develop compensatory adaptive mechanisms. However, the postreoxygenation changes seen after acute hypoxemia parallel those reported in cyanotic patients who undergo reoxygenation during bypass [10, 11]. In contrast to our acute hypoxic model, chronic cyanosis is associated with metabolic adaptations that allow normal aerobic metabolism to persist in the resting state. Studies of mitochondria from chronically cyanotic patients show a 30–40% greater oxidative capability than in the mitochondria of normoxemic myocardium. This finding may, in part, explain the capacity to maintain aerobic metabolism under resting conditions in chronically cyanotic patients despite low oxygen tension [3, 6, 7, 9, 12, 29, 41]. However, this compensatory mechanism is expended readily with stress, as atrial pacing causes myocardial lactate production, indicating ischemia with a shift toward anaerobic metabolism [13, 42]. This metabolic shift may also occur in cyanotic patients during the stresses of daily life, such as exercise, emotional upset, and tachycardia, and become compounded during anoxic spells. Compared to the acute hypoxic animal, the chronically cyanotic infant may, therefore, be susceptible to a reoxygenation, as well as an ischemic (reperfusion) injury.

Our in vivo model of hypoxemia was an acute preparation, consequently, the aforementioned mitochondrial adaptions were absent. The assurance of normal oxygen delivery by increasing coronary perfusion and hematocrit level during hypoxemia avoided ischemia and ensured a compensated state of hypoxemia that may be relevant to the chronically cyanotic condition. These experimental findings suggest that endogenous antioxidant defense mechanisms are weakened by hypoxemia followed by reoxygenation, therefore rendering the

cyanotic myocardium more susceptible to subsequent intraoperative stress during surgical (cardioplegic) ischemia. This may explain clinical reports showing that cyanotic hearts are more vulnerable than noncyanotic hearts to ischemia/reperfusion damage despite comparable cardioplegic protection and shorter ischemic times [2, 3, 7–9, 43, 44]. The clinical findings of accelerated adenosine triphosphate depletion, lactate accumulation, and myocardial depression provide confirmation that chronic cyanosis predisposes the myocardium to energy depletion and postischemic contractile dysfunction following cardioplegic arrest [2, 3, 8, 9]. These reports did not, however, consider the possibility that reoxygenation-induced damage before superimposed ischemia contributed to the final biochemical and functional findings. In contrast, our initial experimental studies intentionally excluded any surgical (cardioplegic) ischemia to focus on the potential adverse effects of reinstitution of molecular oxygen to the hypoxemic myocardium [28]. We examined only changes in the heart and lungs. However, since the generation of oxygen radicals occurs systemically, it is almost certain that tissue damage occurred in other organs. Numerous experimental and clinical studies now support the beneficial effects of leukocyte depletion [17, 20, 28, 38, 45–49]. In fact, even partially removing white cells has been found to prevent oxygen radical damage and subsequent lung injury in cyanotic and pulmonary hypertensive infants [38]. Therefore, despite the shortcomings of this acute hypoxic model, it seems likely that the beneficial effects observed in this study will be seen if a normoxia and leukodepletion are used clinically. The avoidance of this injury could be especially important in lesions such as hypoplastic left heart, where any myocardial or pulmonary damage could have severe consequences.

Clinical Studies

Experimental studies from several laboratories have now demonstrated the occurrence of an unintended injury with sudden reoxygenation of the hypoxic heart, which is mediated by oxygen free radicals, and results in significant myocardial depression and pulmonary dysfunction [14, 28, 50, 51]. These adverse effects can be modified by initiating bypass at a lower oxygen concentration, or using white cell filtration. However, these studies used an acute hypoxic model, and as described above, the results may be different in the chronically cyanotic infant. In order to determine the clinical relevance and confirm the validity of our experimental findings, we biopsied myocardial tissue in cyanotic and acyanotic patients before and 10 min after initiating bypass to determine the antioxidant reserve capacity. This allows quantification of oxygen free radical formation during reoxygenation and enables us to compare these results

to numerous experimental studies of acute hypoxia [14, 28, 50–52]. In addition, there appears to be a direct linkage between antioxidant depletion, oxidant damage, and cardiac and pulmonary dysfunction [14, 28, 50, 51]. Antioxidant reserve capacity also predicts the ability of the heart to withstand a subsequent ischemic challenge. Normal hearts with abundant antioxidants develop only minor functional impairment after aortic clamping, whereas hearts with a limited antioxidant reserve capacity exhibit marked contractile depression after cardioplegic arrest [8, 10, 11, 50, 53].

There was no difference in the prebypass antioxidant reserve capacity between cyanotic and acyanotic hearts. This parallels our experimental findings after acute hypoxia [28]. Initiating bypass in low-risk acyanotic infants (atrial or ventricular septel defect) caused minimal change in the antioxidant reserve capacity, inferring that in the absence of hypoxia, only a small quantity of oxygen free radicals are generated. This small increase in antioxidant reserve capacity in acyanotic hearts may reflect the high levels of oxygen (100%) used in the bypass circuit, as recent evidence demonstrates that even a PO_2 of 185 mm Hg may be detrimental [35]. Alternatively, CPB has been shown to produce an inflammatory reaction characterized by activation of numerous pathways, some of which may result in the generation of oxygen radicals [2]. In contrast, abrupt reoxygenation of cyanotic infants resulted in a significant depletion of endogenous tissue antioxidants (fig. 10). This suggests that abrupt reoxygenation of chronically hypoxemic infants generates abundant oxygen free radicals, since prebypass endogenous tissue stores of antioxidants were not different between acyanotic and cyanotic hearts. This, again, parallels our experimental studies [28].

Cyanotic infants reoxygenated using a PO_2 of 400–550 mm Hg (FiO_2 100%) had the greatest loss of myocardial antioxidant reserve capacity (highest MDA formation) indicating the largest exposure to oxygen free radicals (fig. 11). This phenomenon of oxidant damage with abrupt reoxygenation supports previous studies in cyanotic patients undergoing operative repair [10, 11, 23]. Although both cyanotic infants and acute hypoxic animals demonstrate a reoxygenation injury when exposed to 100% oxygen, the generation of MDA is 4–6 times greater in cyanotic infants [28, 52]. This implies a greater production of oxygen free radicals with reoxygenation after chronic cyanosis. One explanation may be that cyanotic infants can become ischemic during exercise or stress, subjecting them not only to a hypoxic, but also to an ischemic injury [13, 42]. Alternatively, compensatory changes that occur in the cyanotic infant may predispose to the generation of larger amounts of oxygen radicals with the reintroduction of high levels of oxygen. This oxidant injury probably explains why ventricular function is often depressed in cyanotic infants undergoing surgical correction or ECMO, even in the absence of surgical ischemia [8, 22, 23, 54].

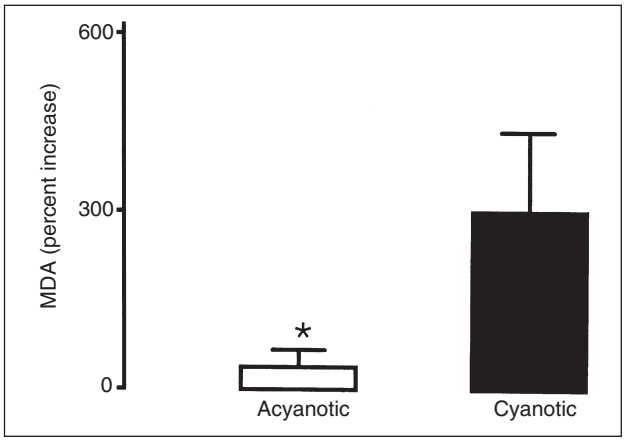


Fig. 10. Percent increase in myocardial tissue antioxidant reserve capacity in acyanotic and cyanotic infants after reoxygenation using CPB. *$p < 0.05$.

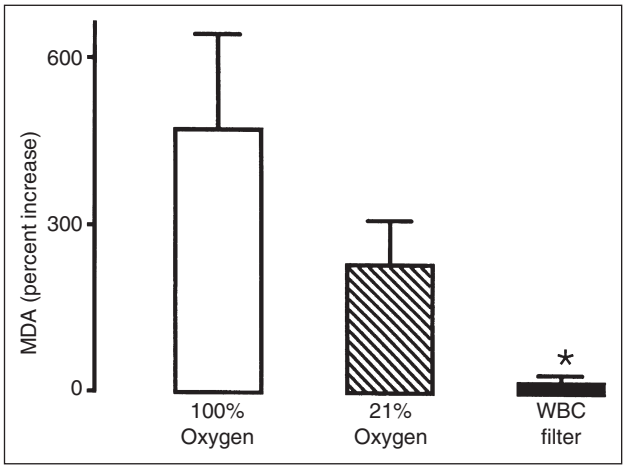


Fig. 11. Percent increase in antioxidant reserve capacity in cyanotic infants after reoxygenation with CPB using 100% oxygen, 21% oxygen, or WBC filtration. *$p < 0.05$.

Compared to cyanotic infants reoxygenated using 100% oxygen, initiating bypass using 21% oxygen reduced the change in antioxidant reserve capacity (fig. 11). The improvement in antioxidant reserve capacity with lower levels of oxygen also mimics the experimental results, but the benefit was less than after acute hypoxia [53]. However, using 21% oxygen to prime the bypass circuit in

cyanotic patients resulted in a PO_2 of 140–155 mm Hg, which was substantially higher than the PO_2 of 80–100 mm Hg used in the experimental study [53]. This may have at least partially accounted for the lack of improvement seen with 21% oxygen in cyanotic infants, as oxygen free radical production and myocardial injury after reoxygenation are proportional to oxygen tension [33, 34]. Since current membrane oxygenators are so efficient, however, it will take oxygen concentrations less than 21% to obtain a PO_2 of 80–100 mm Hg in the bypass prime. These lower levels (PO_2 80–100 mm Hg) have also been shown to improve tissue perfusion during CPB. Therefore a higher oxygen level is probably never needed, as a PO_2 greater than 100–150 mm Hg confers only negligible increase in O_2 content [35].

When neutrophils were reduced by a leukocyte-depleting filter in cyanotic infants, the detrimental effects of sudden reoxygenation were obviated, resulting in preservation of antioxidant reserve capacity (fig. 11). This is once again precisely what was demonstrated in the experimental setting after acute hypoxia, where a lower production of oxygen free radicals correlated with an improvement in myocardial and pulmonary function [28]. This functional improvement also appears to occur clinically, as several studies have documented, as even partially removing white cells lowers postoperative pulmonary vascular resistance and improves cardiac performance following open heart surgery [38, 46–48]. Although no patient reoxygenated with leukocyte-depleted blood had a substantial change in the antioxidant reserve capacity, the generation of oxygen free radicals was further suppressed by using 21% oxygen (fig. 12). Indeed, the antioxidant reserve capacity in these infants was unchanged from baseline values, and even lower than in acyanotic patients, suggesting the effects of lower oxygen levels and white cell filtration are additive.

As in the experimental study, WBC filtration substantially reduced leukocytes both initially, and after 30 min of CPB (fig. 13). Unfortunately, no WBC filter currently on the market is ideal. We chose the Pall BC-1 filter in our initial clinical investigation because it is the most efficient leukocyte filter available, and it worked well in our experimental study [28]. At flows of 500–600 cm^3/min, the BC-1 removes almost all WBCs in one pass, and these flows are adequate for 3 to 4-kg newborns. We kept the filter in the bypass circuit for up to 60 min, without any complications, and the pressure across the filter remained low with no significant change over time. However, if higher flows are utilized, these filters are less efficient, since WBC filters are flow dependent. Furthermore, the BC-1 can only be used up to flows of approximately 600–700 cm^3/min, and may become fully saturated if used for a prolonged time. Therefore, this filter is not applicable in larger infants, and may be problematic with long bypass runs. Instead, a Pall LG-6 filter is needed, as this filter can accommodate flows up to 6 liters/min for a prolonged time. This filter

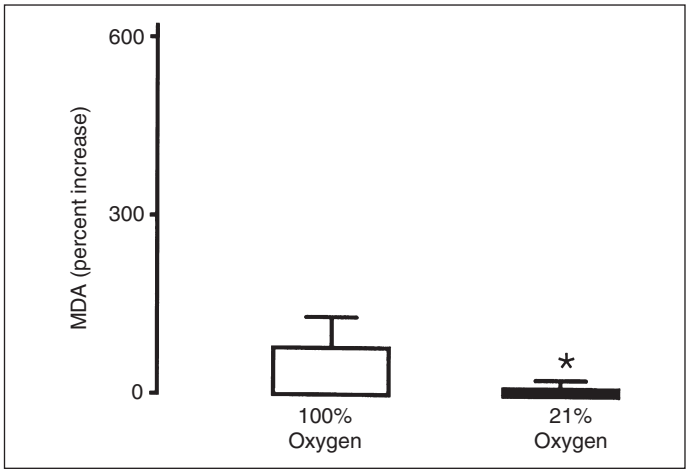


Fig. 12. Percent increase in antioxidant reserve capacity in cyanotic infants reoxygenated using CPB with WBC filtration at either 100% or 21% oxygen. *p $<$ 0.05.

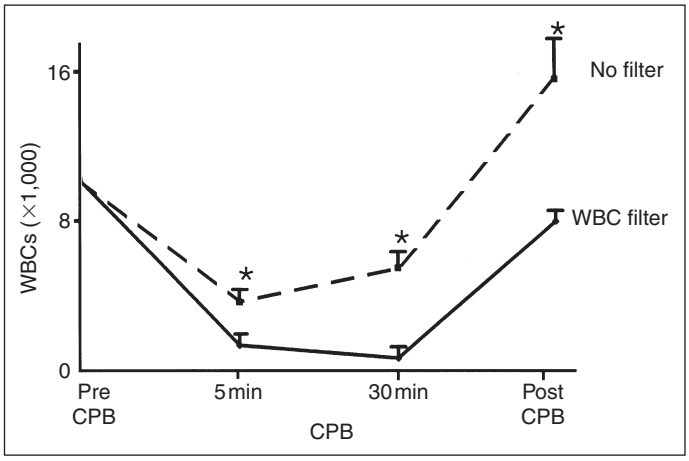


Fig. 13. Total WBC count in pediatric patients undergoing CPB with and without leukofiltration, before bypass, 5 and 30 min after initiating CPB, and post CPB on arrival in the intensive care unit. *p $>$ 0.05.

is less efficient in removing WBCs during the first pass, and instead removes neutrophils slowly over time. The LG-6 filter, however, removes a significant number of WBCs at lower flow rates (1–2 liters/min), since WBC removal is flow dependent. Therefore, the LG-6 filter is still very effective in infants or

small children, and it is the filter we now use clinically. We always pre-filter all blood added to the cardiopulmonary circuit using a Pall RC-400 leukocyte filter. This results in extremely low WBC counts in the bypass prime, which has been shown to reduce oxygen radical formation and improve postoperative pulmonary vascular resistance even in acyanotic infants [17, 38]. I strongly believe blood for the bypass prime should always be leukodepleted, since oxygen free radical formation is greatest with the initial reintroduction of oxygen. This is the most important time to limit WBC exposure. In addition, banked blood contains a large amount of activated WBCs, which can cause damage even in the absence of hypoxia [38]. Inefficient WBC filters, coupled with leukodepletion during times which are less critical, probably explains why some investigators have failed to demonstrate a clinical advantage with WBC filtration, despite overwhelming experimental evidence as to their benefit [38, 46–48]. Clearly, a more efficient inline arterial filter that substantially reduces leukocyte counts in a single pass, over the entire bypass run, would alleviate this problem. However, until such filters are commercially available, clinical improvement will probably only be seen when WBC filters are used in a specific manner, and directed at distinct events. Although there is some concern that leukocyte depletion may increase postoperative infection rates, this has not been reported in over 21,000 patients, and indeed, there is evidence that it may lower the risk of infection [55–57]. Furthermore, the WBC count in all patients was back to pre-bypass levels by arrival to the intensive care unit.

Current Clinical Practice

Based on this extensive experimental and clinical infrastructure, we now use a normoxic bypass strategy combined with leukodepletion in all cyanotic or high-risk normoxic patients (table 1). Bypass is initiated at 21% FiO_2, and the FiO_2 increased to 30–50% slowly as needed over the next 10–20 min. We only resort to higher oxygen levels (FiO_2 100%) if we require a period of low flow or deep hypothermic circulatory arrest, as the increased oxygen content has been shown to be important for neuroprotection [58]. If a blood prime is used, it is always washed and leukodepleted (Pall RC-400), and a Pall LG-6 leukodepleting filter placed in the arterial line for the entire procedure.

To assess the clinical efficacy of this approach, we retrospectively examined all patients undergoing a Norwood procedure at our institution between July 1, 1996 and March 31, 2000. There were 72 patients, 41 with a diagnosis of hypoplastic left heart syndrome (HLHS), and 31 with a variant (HLHV) of hypoplastic left heart syndrome. The overall survival was 78% (76% HLHS,

Table 1. Limiting the reoxygenation injury in clinical practice–bypass protocol

Wash and leukodeplete blood prime (Pall RC-400 filter)
Inline arterial filter (Pall LG-6 filter)
Initiate bypass using normoxic management (FiO_2 21%)

Table 2. Norwood operation clinical results

Seventy-two patients (July 1, 1996 to March 31, 2000)
41 HLHS, 31 HLHV
Age 7 days (range 2–44 days)
Weight 3,110 g (range 1,680–5,195 g)
Overall perioperative survival 78% (56/72 patients)
HLHS 76%, HLHV 80%
HLHS right ventricular function
Fractional shortening (echocardiogram); preoperative 37 ± 10% vs. postoperative 35 ± 11%
Ejection fraction (angiogram) at time of Glenn shunt 57 ± 7%
Prior perioperative survival 53% (January 1, 1993 to June 30, 1996, 38 patients)

81% HLHV). More importantly, there was excellent preservation of myocardial function, both initially, as assessed by echocardiogram, and several months later, when evaluated by angiogram prior to the Glenn procedure (table 2). Preservation of myocardial function suggests that the reoxygenation injury was avoided, thus allowing the heart to better tolerate the period of surgical ischemia needed for repair. This is in contrast with other reports which often describe depressed myocardial function following successful repair of cyanotic lesions [2, 3, 8]. Indeed, the surgical survival in the 38 patients undergoing a Norwood procedure in the 2 years prior to instituting this strategy (January 1, 1993 to June 30, 1996) was only 53%. The only major changes we made between these two time intervals was using a leukodepleted normoxic strategy to initiate bypass, and infusing a continuous modified (nonpotassium) cardioplegic solution during the period of myocardial ischemia. I acknowledge that this change in our myocardial protection strategy, or other unknown factors, might have accounted for the increased survival and improved postoperative cardiac function. However, despite the lack of a control group, I believe these results, as well as the extensive experimental infrastructure, support the safety and efficacy of this approach.

Cardioplegia Solutions

Cardioplegic solutions have been used in adults to resuscitate the heart after a reperfusion injury, and so, they might also be able to repair the damage caused by reoxygenation [5, 59]. However, the injury caused by hypoxia/reoxygenation is more severe than the injury from ischemia/reperfusion. Therefore, in addition to testing an aspartate/glutamate cardioplegic solution, which effectively resuscitates the ischemic heart, we tested solutions enriched with *L*-arginine or leukodepleted, since these strategies have been shown to further improve cardioplegic protection [51, 60].

Ischemia and hypoxia both cause endothelial cell dysfunction with impaired release of NO during reperfusion (reoxygenation) [25, 26, 50, 61, 62]. This may predispose the tissue to an exacerbated injury as a result of unscavenged oxygen free radicals, vasoconstriction, and increased WBC and platelet adherence. In the presence of the enzyme NO synthase, *L*-arginine combines with oxygen to produce NO and citrulline [61, 63, 64]. NO then reacts with the vascular endothelium resulting in vasorelaxation, decreased platelet and WBC adherence, reduced chemotaxis of WBCs, and neutralization of superoxide radicals. However, *L*-arginine (NO) also produces the toxic oxygen free radical peroxynitrite, and so under certain conditions, it can be detrimental [18, 63]. A WBC filter does not act directly on the vascular endothelium or cause vasodilatation. It therefore allows quantification of the contribution of WBCs in mediating the reperfusion injury independent of other factors (i.e., vasodilation).

Following a hypoxic-reoxygenation insult, hearts protected with Asp/Glut blood cardioplegia alone sustained a significant oxygen-free-radical-mediated injury resulting in vascular dysfunction, mitochondrial damage, and depressed myocardial function (fig. 14). In contrast, oxygen free radical production was equally reduced if the cardioplegia was enriched with *L*-arginine, or passed through a WBC filter. This allowed the hypoxic-ischemic injury to be repaired, resulting in complete preservation of vascular, metabolic, and myocardial function (fig. 14). Compared to *L*-arginine, use of leukocyte filtration resulted in similar levels of oxygen free radical production, myeloperoxidase activity, as well as metabolic and functional recovery [60]. This implies that WBCs are responsible for the generation of the majority of oxygen free radicals during reperfusion, as well as that the ability of *L*-arginine to repair the hypoxic insult is primarily due to inhibition of WBC adherence.

Coronary vascular resistance was measured with each cardioplegic infusion to determine the effect each strategy had on vascular function (fig. 15). The higher coronary vascular resistance with Asp/Glut blood cardioplegia alone implies vascular dysfunction secondary to endothelial cell injury. Conversely, the lower coronary vascular resistance with *L*-arginine or WBC filtration implies

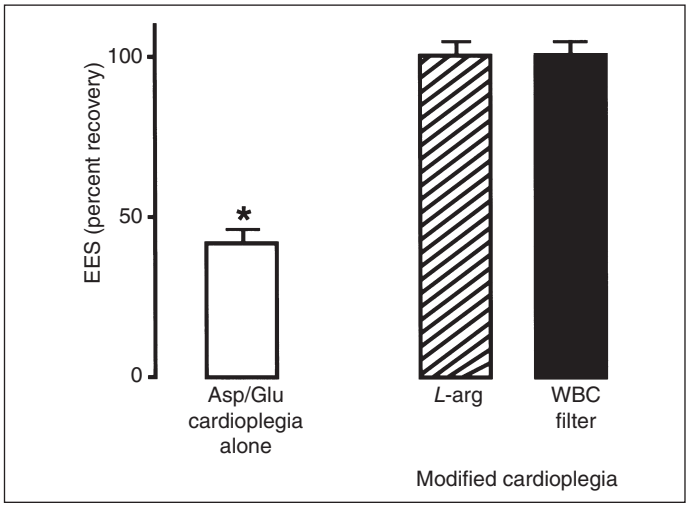


Fig. 14. Post-CPB recovery of LV systolic function as measured by end systolic elastance (EES) and expressed as percentage of control (baseline), in animals protected with aspartate/glutamate (Asp/Glut) blood cardioplegia alone, enriched with *L*-arginine, or leukodepleted (WBC filter). *$p < 0.05$.

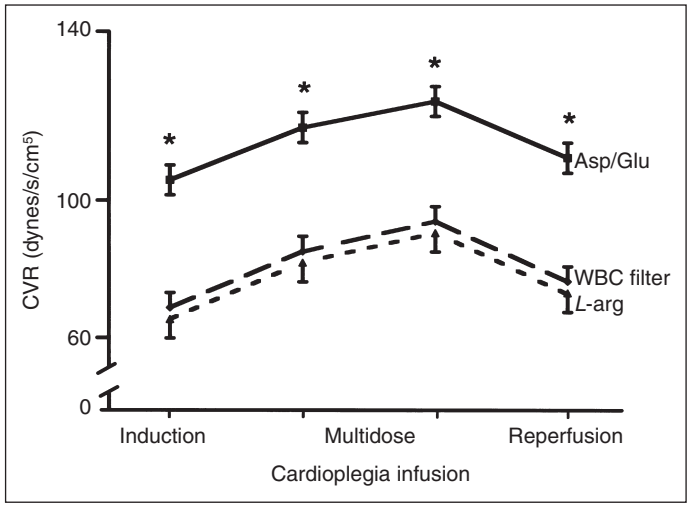


Fig. 15. Coronary vascular resistance (CVR) measured during each cardioplegic infusion once the flow and cardioplegic pressures were stable, in animals protected with aspartate/glutamate (Asp/Glut) blood cardioplegia alone, enriched with *L*-arginine, or leukodepleted (WBC filter). *$p < 0.05$.

avoidance or repair of this injury with preservation of vascular function. Because a WBC filter does not cause vasodilatation, the similar coronary vascular resistance in these two groups suggests that *L*-arginine is acting primarily to preserve vascular function through inhibition of WBCs, and not as vasodilators. I cannot prove that *L*-arginine and WBC filtration work via an identical pathway, because we did not specifically examine the mechanism of action with each intervention. It is possible that *L*-arginine limits the reperfusion injury via a non-WBC-dependent mechanism, or by a combination of effects, since it also prevents platelet adherence and induces vasodilatation. However, because the vascular, metabolic, and functional recoveries are identical in each group, and one of the primary effects of *L*-arginine (NO) is to prevent adherence of activated WBCs, this is probably responsible for most of the beneficial effects.

This study does not allow us to determine whether combining a WBC filter with a pharmacologic agent (*L*-arginine) would further improve results, because hearts receiving either *L*-arginine or leukodepletion recovered completely. However, because of differences between these modalities, it is possible that they should be used together. For instance, *L*-arginine is not as effective when given cold, whereas WBC filters are less affected by temperature. The optimal dosage of *L*-arginine is unknown in humans under various pathologic conditions. Therefore, if a low dose is used for safety, it may not be as effective. Some WBCs escape filtration, and leukocyte filters become less efficient with greater volumes or higher flow rates. A chemical (pharmacologic) blocker may help prevent adhesion of these unfiltered leukocytes. *L*-arginine promotes vasodilation, and limits platelet adherence [25, 61]. Increased vascular resistance may limit cardioplegic distribution, and platelet deposition results in capillary plugging with release of potent vasoconstrictors. In contrast, the Pall BC-1 leukocyte-depleting filter only slightly reduces platelet counts. The above factors probably explain why Hiramatsu et al. [65] demonstrated improvement when WBC filtration and *L*-arginine were combined. Using a pharmaceutical agent along with a WBC filter may, therefore, further improve myocardial protection in cyanotic children.

In conclusion, significant advances have been made in the technical performance of operations for congenital heart disease, but few investigations have examined the conduct of cardiopulmonary bypass in producing myocardial and pulmonary dysfunction. Postoperative organ dysfunction, however, remains problematic, especially in cyanotic infants. These studies provide direct evidence that an unintended oxygen-free-radical-mediated injury occurs in cyanotic infants with the initiation of bypass, resulting in myocardial and pulmonary damage. They further show that this reoxygenation injury can be reduced by using a bypass strategy which incorporated normoxia and WBC filtration, and that a

leukodepleted cardioplegia solution can repair this injury. Incorporating these strategies into the operative management will allow surgeons to limit damage in these high-risk infants, minimizing postoperative organ dysfunction, leading to a reduction in morbidity and mortality. Hopefully, we can completely avoid reoxygenation damage in the future as we learn more about the pathophysiology of hypoxia and reoxygenation, resulting in the development of better strategies for the conduct of bypass. However, the more subtle forms of the cardiac and pulmonary damage that still exist will not be known until we develop both the impetus and simplified methodology for routine study of ventricular and pulmonary performance months and years after operation.

References

1 Bull C, Cooper J, Stark J: Cardioplegia protection of the child's heart. J Thorac Cardiovasc Surg 1984;88:287–293.

2 Castaneda AR, Jonas RA, Mayer JE Jr, Hanley FL: Myocardial preservation in the immature heart; in Castaneda AR, Jonas RA, Mayer JE Jr, Hanley FL (eds): Cardiac surgery of the neonate and infant. Philadelphia, Saunders, 1994, pp 41–54.

3 Hammon JW Jr: Myocardial protection in the immature heart. Ann Thorac Surg 1995;60: 839–842.

4 Romero TE, Friedman WF: Limited left ventricular response to volume overload in the neonatal period: A comparative study with the adult animal. Pediatr Res 1979;13:910–915.

5 Buckberg GD, Allen BS: Myocardial protection management during adult cardiac operations; in Baue AE, Geha AS, Hammond GL, Laks H, Naunheim KS (eds): Glenn's Thoracic and Cardiovascular Surgery, ed 6. Stamford, Appleton & Lange, 1995, pp 1653–1687.

6 Yee ES, Ebert PA: Effect of ischemia on ventricular function, compliance, and edema in immature and adult canine hearts. Surg Forum 1979;30:250–252.

7 Fujiwara T, Kurtts T, Anderson W, Heinle J, Mayer JE Jr: Myocardial protection in cyanotic neonatal limbs. J Thorac Cardiovasc Surg 1988;96:700–710.

8 Del Nido PJ, Mickle DAG, Wilson GJ, Benson LN, Weisel RD, Coles JG, et al: Inadequate myocardial protection with cold cardioplegic arrest during repair of tetralogy of Fallot. J Thorac Cardiovasc Surg 1988;95:223–229.

9 Silverman N, Kohler J, Levitsky S, Pavel D, Fang R, Feinberg H: Chronic hypoxemia depresses global ventricular function and predisposes to depletion of high energy phosphates during cardioplegic arrest: Implications for surgical repair of cyanotic congenital heart defects. Ann Thorac Surg 1984;37:304–308.

10 Teoh KH, Mickle DAG, Weisel RD, Li R, Tumiati L, Coles JG: Effect of oxygen tension and cardiovascular operations on the myocardial antioxidant enzyme activities in patients with tetralogy of Fallot and aorta-coronary bypass. J Thorac Cardiovasc Surg 1992;104:159–164.

11 Del Nido PJ, Mickle DAG, Wilson G, Benson LN, Coles JG, Trusler GA: Evidence of myocardial free radical injury during elective repair of of tetralogy of Fallot. Circulation 1987;76:174–179.

12 Lee J, Halloran K, Taylor J, et al: Coronary flow and myocardial metabolism in newborn lambs: Effect of hypoxia and acidemia. Am J Physiol 1973;224:1381.

13 Graham TP Jr, Erath HG Jr, Buckspan GS, Fisher RD: Myocardial anaerobic metabolism during isoprenaline infusion in a cyanotic animal model: Possible cause of myocardial dysfunction in cyanotic congenital heart disease. Cardiovasc Res 1979;13:401–406.

14 Ihnken K, Morita K, Buckberg GD, Matheis G, Sherman MP, Allen BS, et al: Studies of hypoxemic/reoxygenation injury: Without aortic clamping. II. Evidence for reoxygenation damage. J Thorac Cardiovasc Surg 1995;110:1171–1181.

15 Feldbaum DM, Kohman LJ, Veit LJ: Recovery of hypoxic neonatal hearts after cardioplegic arrest. Cardiovasc Res 1993;27:1123–1126.
16 Breda MA, Drinkwater DC, Laks H, Bhuta S, Corno A: Prevention of reperfusion injury in the neonatal heart with leukocyte-depleted blood. J Thorac Cardiovasc Surg 1989;97:654–665.
17 Bando K, Pillai R, Cameron DE, Brawn JD, Winkelstein JA, Hutchins GM, et al: Leukocyte depletion ameliorates free radical-mediated lung injury after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;99:873–877.
18 Matheis G, Sherman P, Buckberg G, Haybron D, Young H, Ignarro L: Role of *L*-arginine-nitric oxide pathway in myocardial reoxygenation injury. Am J Physiol 1992;262:H616–H620.
19 Lucchese B: Myocardial ischemia, reperfusion and free radical injury. Am J Cardiol 1990;65:14I–23I.
20 Byrne J, Appleyard R, Lee C, et al: Controlled reperfusion of the regionally ischemic myocardium with leukocyte-depleted blood reduces stunning, the no-reflow phenomenon, and infarct size. J Thorac Cardiovasc Surg 1992;103:66–72.
21 Kraemer R, Mullane K: Neutrophils delay functional recovery of the post hypoxic heart of the rabbit. J Pharmac Exp Ther 1989;251:620–626.
22 Hirschl R, Heiss K, Bartlett R: Severe myocardial dysfunction during extracorporeal membrane oxygenation. J Pediatr Surg 1992;27:48–53.
23 Martin G, Short B, Abbott C, O'Brien A: Cardiac stun in infants undergoing extracorporeal membrane oxygenation. J Thorac Cardiovasc Surg 1991;101:607–611.
24 Zweir J, Kuppusamy P, Lutty G: Measurement of free radical generation in reoxygenated human endothelial cells. Circulation 1990;82:III–701.
25 Boyle ED Jr, Pohlman TH, Cornejo CJ, Verrier ED: Ischemia-reperfusion injury. Ann Thorac Surg 1997;64:S24–S30.
26 Verrier ED, Boyle ED Jr: Endothelial cell injury in cardiovascular surgery: An overview. Ann Thorac Surg 1997;64:S2–S8.
27 Haybron DM, Matheis GF, Buckberg GD, Sherman MP, Byrns R, Ignarro LJ: Hypoxic pulmonary hypertension in the neonatal piglet is related to decreased intravascular endothelium-derived nitric oxide. Surg Forum 1991;42:285–287.
28 Bolling KS, Halldorsson A, Allen BS, Rahman SK, Wang T, Kronon M, et al: Prevention of the hypoxic/reoxygenation injury using a leukocyte depleting filter. J Thorac Cardiovasc Surg 1997; 113:1081–1090.
29 Lupinetti FM, Wareing TH, Huddleston CB, Collins JC, Boucek RJ Jr, Bender HW Jr, Hammon JW Jr: Pathophysiology of chronic cyanosis in a canine model. J Thorac Cardiovasc Surg 1985;90:291–296.
30 Mullane K, Read N, Salmon J, Moncada S: Role of leukocyte in acute myocardial infarction in anesthetized dogs: Relationship to myocardial salvage by anti-inflammatory drugs. Circ Res 1983; 53:584–591.
31 Tauber A, Babior B: Neutrophil oxygen reduction: The enzymes and the products. Adv Free Radic Biol 1985;1:265–272.
32 Harlan J: Leukocyte-endothelial interactions. Blood 1985;65:513–525.
33 Gauduel Y, Menasche P, Duvelleroy M: Enzyme release and mitochondrial activity in reoxygenated cardiac muscle: Relationship with oxygen-induced lipid perioxidation. Gen Physiol Biophys 1989;8:327–340.
34 Hearse D, Humphrey S, Bullock G: The oxygen paradox and the calcium paradox: Two facets of the same problem? J Mol Cell Cardiol 1978;10:641–668.
35 Joachimsson P, Sjoberg F, Forsman M, Johansson M, Casimir Ahn H, Rutberg H: Adverse effects of hyperoxemia during cardiopulmonary bypass. J Thorac Cardiovasc Surg 1996;112: 812–819.
36 Engler R, Schmid-Schoebein G, Pavelec R: Role of leukocyte capillary plugging in preventing myocardial reperfusion. Circulation 1981;64:IV–138.
37 Jolly S, Kane W, Hook B, Abrams G, Kunkel S, Lucchesi B: Reduction of myocardial infarct size by neutrophil depletion: Effect of duration of occlusion. Am Heart J 1988;112: 682–690.
38 Komai H, Yamamoto F, Tanaka K, Yagihara T, Kawashima Y: Prevention of lung injury during open heart operations for congenital heart defects. Ann Thorac Surg 1994;57:134–140.

39 Rowe G, Manson N, Caplan M, Hess M: Hydrogen peroxide and hydroxyl radical mediation of activated leukocyte depression of cardiac sarcoplasmic reticulum: Participation of the cycloxygenase pathway. Circ Res 1983;53:584–591.
40 Swindle N: Porcine models in surgical research: An overview; in Tumbleson M (ed): Advances in Swine in Biomedical Research. Plenum Press, New York, 1986, pp 235–241.
41 Murashita T, Borgers M, Hearse DJ: Developmental changes in tolerance to ischemia in the rabbit heart: Disparity between interpretations of structural, enzymatic and functional indices of injury. J Mol Cell Cardiol 1992;24:1143–1154.
42 Boucek RJ Jr, Kasselberg AG, Boerth RC, Parrish MD, Graham TP Jr: Myocardial injury in infants with congenital heart disease: Evaluation by creatine kinase MB isoenzyme analysis. Am J Cardiol 1982;50:129–135.
43 Bolling KS, Kronon M, Allen BS, Ramon S, Wang T, Hartz R, et al: Myocardial protection in normal and hypoxically stressed neonatal hearts: The superiority of hypocalcemic versus normocalcemic blood cardioplegia. J Thorac Cardiovasc Surg 1996;112:1193–1201.
44 Bolling KS, Kronon M, Allen BS, Wang T, Ramon S, Feinberg H: Myocardial protection in normal and hypoxically stressed neonatal hearts: The superiority of blood versus crystalloid cardioplegia. J Thorac Cardiovasc Surg 1997;113:994–1005.
45 Wilson IC, Gardner TJ, DiNatale BS, Gillinov AM, Curtis WE, Cameron DE: Temporary leukocyte depletion reduces ventricular dysfunction during prolonged postischemic reperfusion. J Thorac Cardiovasc Surg 1993;106:805–810.
46 Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W: Leukocyte depletion results in improved lung function and reduced inflammatory response after cardiac surgery. J Thorac Cardiovasc Surg 1996;112:494–500.
47 Morioka K, Muraoka R, Chiba Y, Ihaya A, Kimura T, Noguti H, et al: Leukocyte and platelet depletion with a blood cell separator: Effects on lung injury after cardiac surgery with cardiopulmonary bypass. J Thorac Cardiovasc Surg 1996;111:45–54.
48 Chiba Y, Morioka K, Muraoka R, Ihaya A, Kimura T, Uesaka T, et al: Effects of depletion of leukocytes and platelets on cardiac dysfunction after cardiopulmonary bypass. Ann Thorac Surg 1998; 65:107–113.
49 Wilson IC, DiNatale JM, Gillinov AM, Curtis WE, Cameron DE, Gardner TJ: Leukocyte depletion in a neonatal model of cardiac surgery. Ann Thorac Surg 1993;55:12–19.
50 Buckberg GD: Studies of hypoxemic/reoxygenation injury. I. Linkage between cardiac function and oxidant damage. J Thorac Cardiovasc Surg 1995;110:1164–1170.
51 Ihnken K, Morita K, Buckberg GD, Sherman MP, Young HH: Studies of hypoxemic/reoxygenation injury: Without aortic clamping. III. Comparison of the magnitude of damage by hypoxemia/reoxygenation versus ischemia/reperfusion. J Thorac Cardiovasc Surg 1995;110:1182–1189.
52 Allen BS, Rahman SK, Ilbawi M, Feinberg H, Bolling KS, Kronon M: The detrimental effects of cardiopulmonary bypass in cyanotic infants: Preventing the reoxygenation injury. Ann Thoracic Surg 1997;64:1381–1388.
53 Morita K, Ihnken K, Buckberg G, Sherman MP, Young HH: Studies of hypoxemic/reoxygenation injury: Without aortic cross clamping. IX. Importance of avoiding perioperative hyperoxemia in the setting of previous cyanosis. J Thorac Cardiovasc Surg 1995;110:1235–1244.
54 Rocchini AP, Keane JF, Cantaneda AR, Nadas AS: Left ventricular function following attempted surgical repair of tetralogy of Fallot. Circulation 1978;57:798–802.
55 Ortolano J: Pall Corporation, Glencove, N. Y. pers. commun. 1997.
56 Jewett PH, Kissmeyer-Nielsen P, Wolff B, Qvist N: Randomised comparison of leucocyte-depleted versus buffy-coat-poor blood transfusion and complications after colorectal surgery. Lancet 1996;348:841–845.
57 Bowden AR, Slichter JS, Sayer M, Weisdorf D, Cays M, Schoch G, et al: A comparison of filtered leukocyte-reduced and cytomegalovirus (CMV) seronegative blood products for the prevention of transfusion-associated CMV infection after marrow transplant. Blood 1995;86:3598–3603.
58 Nollert G, Nagashima M, Bucerius J, Shin'oka T, Lidov HG, du Plessis A, et al: Oxygenation strategy and neurologic damage after deep hypothermic circulatory arrest. II. Hypoxic versus free radical injury. J Thorac Cardiovasc Surg 1999;117:1172–1179.

59 Allen BS, Okamoto F, Buckberg GD, Bugyi HI, Young H, Leaf J, et al: Studies of controlled reperfusion after ischemia. XV. Immediate functional recovery after 6 hours of regional ischemia by careful control of conditions of reperfusion and composition of reperfusate. J Thorac Cardiovasc Surg 1986;92(suppl):621–635.
60 Kronon MT, Allen BS, Halldorsson A, Rahman SK, Wang T, Ilbawi M: *L*-Arginine, prostaglandin, and white cell filtration equally improve myocardial protection in stressed neonatal hearts. J Thorac Cardiovasc Surg 1999;118:665–673.
61 Mizuno A, Baretti R, Buckberg GD, Young H, Vinten-Johansen J, Ma X, et al: Endothelial stunning and myocyte recovery after reperfusion of jeopardized muscle: A role of *L*-arginine blood cardioplegia. J Thorac Cardiovasc Surg 1997;113:379–389.
62 Boyle EMJ, Pohlman TH, Johnson M, Verrier ED: Endothelial cell injury in cardiovascular surgery: The systemic inflammatory response. Ann Thorac Surg 1997;63:277–284.
63 Kronon M, Allen BS, Halldorsson A, Rahman S, Wang T, Ilbawi M: Dose dependency of *L*-arginine in neonatal myocardial protection: The nitric oxide paradox. J Thorac Cardiovasc Surg 1999;118:665–672.
64 Morita K, Sherman MP, Buckberg GD, Ihnken K, Matheis G, Young HH, Ignarro LJ: Studies of hypoxemic/reoxygenation injury: Without aortic clamping. V. Role of the *L*-arginine-nitric oxide pathway: The nitric oxide paradox. J Thorac Cardiovasc Surg 1995;110:1200–1211.
65 Hiramatsu T, Forbess J, Miura T, Nomura F, Mayer JE Jr: Additive effects of *L*-arginine infusion and leukocyte depletion on recovery after hypothermic ischemia in neonatal lamb hearts. J Thorac Cardiovasc Surg 1995;110:172–179.

Bradley S. Allen, MD, The Heart Institute for Children,
Hope Children's Hospital, 4440 West 95th Street, Oak Lawn, IL 60453 (USA)
Tel. +1 708 346 3029, Fax +1 708 346 4068, E-Mail bradallen@thic.com

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 135–146

Blood Cardioplegia and Reperfusion

Juergen Martin

Division of Cardiovascular Surgery, Albert Ludwig University, Freiburg, Germany

Role of Neutrophils

Several experimental and clinical studies have proven the role of neutrophils in the pathophysiology of myocardial ischemia and reperfusion [1–10]. Neutrophil activation occurs early during myocardial ischemia and precedes the appearance of histological tissue injury. Following myocardial ischemia, reperfusion markedly enhances the infiltration of neutrophils into the ischemic heart muscle [8, 11].

Adhesion of neutrophils is the initiating step of neutrophil-mediated cellular damage. Enhanced neutrophil adherence during ischemia and reperfusion is due to changes in neutrophils and endothelial cells [12].

Leukocyte Activation

Ischemia itself induces a more immediate polymorphonuclear leukocyte response and activates granulocytes and the complement cascade [1]. Production of additional chemoattractants by activated neutrophils amplifies the initial inflammatory response. Neutrophils exposed to chemoattractants become more spherical and express a glycoprotein on their surface that promotes adhesion, aggregation, and chemotaxis [13]. Chemotactic factors cause an increase in cytosolic calcium, leading to activation of phospholipases and generation of arachidonate products from cyclooxygenase and lipoxygenase pathways. The release of leukotrienes enhances vascular smooth muscle tone, potentiates platelet aggregation, promotes endothelial permeability, and may also modulate proteolytic enzyme activity. Neutrophil activation during reperfusion is accompanied by a greatly enhanced oxygen uptake resulting in the production of large quantities of reactive oxygen species (respiratory burst), i.e. superoxide anion, hydrogen peroxide, hydroxyl radical, hypochlorous acid, and chloramine [14].

Due to previous ischemia, the antioxidative capacity of the cells is depleted [15]. In addition to their direct effects, oxygen free radicals generate a potent chemotactic factor for neutrophils and thereby create a positive feedback cycle for the generation of more oxygen free radicals [16].

Neutrophil degranulation and free radical release permit an unchecked activity of proteolytic enzymes on endothelial and myocyte membranes. Myeloperoxidase present in neutrophils acts to convert hydrogen peroxide into hypochlorous acid (HOCl), which reacts with low-molecular-weight amines to form toxic chloramines that are lipid soluble and have long half-lives. These chloramines are particularly toxic because of their ability to dissolve in biologic membranes; they cause additional membrane damage through lipid peroxidation [17].

Furthermore, the onset of cardiopulmonary bypass (CPB) is associated with an acute inflammatory response and may enhance neutrophil-mediated myocardial damage. Expression of the neutrophil adhesion molecules CD11b and CD11c is increased [18]. Hypothermia delays but does not prevent the expression of these adhesion molecules.

Endothelial Cell Function and 'Endothelial Stunning'

Intact endothelial cells secrete a variety of compounds to maintain microcirculatory flow, i.e. plasminogen activator, antithrombin III, prostacyclin, adenosine, and nitric oxide. Ischemia significantly inhibits the release of these substances. Hypoxic endothelial cells produce large amounts of endothelin, a potent vasoconstrictor with a long half-life. Reversible impairment of endothelial function after myocardial ischemia, so-called 'microvascular or endothelial stunning' has been observed in numerous studies [19, 20]. Endothelial stunning plays an important role in ischemic and reperfusion injury and has been underestimated in previous preservation strategies [21, 22]. We have to consider endothelial stunning and develop efforts to reduce this injury during myocardial protection [23]. The role of plasma and neutrophils in endothelial stunning has been demonstrated in an isolated rabbit heart model [24]. After 30 min of global ischemia and reperfusion with autologous polymorphonuclear leukocytes and plasma severe depression of coronary flow reserve was observed.

Endothelial cells contain large quantities of adenosine. Due to their capacity to release and take up adenosine, they are responsible for the maintenance of constant adenosine plasma levels. Adenosine has been shown to have broad-spectrum cardioprotective effects by acting through multiple mechanisms on neutrophils, endothelium, and myocytes [25, 26]. This nucleotide is a potent coronary arteriolar vasodilator and important regulator of coronary flow. Berne et al. [27] described the key role of adenosine in the autoregulation of coronary blood flow. Moreover, due to stimulation of myocardial A1 receptors, adenosine

activates ATP-sensitive potassium (K_{ATP}) channels and attenuates myocardial stunning [28]. Applied as an adjunct to standard cold blood cardioplegia in hearts exposed to 30 min of normothermic ischemia, adenosine reversed the postischemic systolic dysfunction [29]. Furthermore, adenosine is an important modulator of neutrophil function as it reduces superoxide generation by stimulated neutrophils and adherence of activated neutrophils to endothelial cells [30, 31].

Basally released nitric oxide exerts a cardioprotective effect via its inhibition of neutrophil activities. Loss of endogenous nitric oxide due to endothelial cell injury may occur during myocardial ischemia and reperfusion. Sato et al. [32] could show that blockade of endogenous nitric oxide by *L*-nitro-arginine as an additive to blood cardioplegic solution augments postischemic injury mediated by polymorphonuclear neutrophils.

Ischemic endothelial cells express membrane receptors for immunoglobulin, complement fragments that promote adherence, and adhesion molecules [2, 6]. Reperfusion with normal blood after 2 or more hours of myocardial ischemia causes extensive cellular membrane and mitochondrial damage with accompanying failure to restore contractility [33]. The severe depression of contractile protein function may be due to the release of cytotoxic agent from the granulocytes in the capillary bed.

Leukocyte Plugging and the 'No-Reflow' Phenomenon

Reperfusion after prolonged myocardial ischemia may result in incomplete restoration of blood flow, the no-reflow phenomenon. The failure of microcirculation is associated with capillary obstruction by neutrophil plugging and endothelial cell protrusion. Complement components and other metabolites of ischemic myocardium cause large polymorphonuclear leukocytes to become stiffer and less capable of smoothly traversing capillaries. These granulocytes become more adherent to endothelium and to one another, which potentially leads to plugging of capillaries, especially when regional perfusion pressure is low during ischemia [34–36].

Following 3 h of myocardial ischemia, up to 27% of capillaries were occluded by accumulating neutrophils. Leukocyte plugging could be completely prevented by reperfusion with leukocyte-depleted blood [37].

Clinical Findings Indicating the Role of Leukocytes during Myocardial Ischemia and Reperfusion

There is some evidence that neutrophil-mediated reperfusion injury plays a role in elective coronary bypass surgery. During coronary artery bypass operations a reduction of neutrophil count in the coronary sinus as compared to the arterial blood could be observed as a result of neutrophil trapping within the

myocardium [38]. In addition, a difference in transmyocardial elastase plasma concentration due to neutrophil degranulation appeared during reperfusion of the myocardium [7].

Furthermore, a beneficial effect could be observed after inhibition of neutrophil function with anti-inflammatory drugs or antibodies limiting neutrophil adhesion [10, 39].

Technical and Biological Properties of Leukocyte Filters

Leukocyte filters consist of two different filtration units. A 40-μm lockwoven polyester screen is capable of retaining gaseous and particulate microemboli. The second filter unit contains a polyester monofilament woven mesh. The fine polyester fibers have a high affinity for leukocytes [40]. Due to permanent adherence to these fibers the leukocytes are removed from circulating blood.

The effectiveness of leukocyte filters depends on the flow rate and the total volume of blood. Heggie et al. [41] tested the Pall leukocyte-depleting blood cardioplegia filter BC-1 in the clinical situation and found a very low resistance to flow with a mean pressure drop of 10.8 mm Hg at a mean flow rate of 315 ml/min The filter removed 70% of leukocytes, 11.3% of platelets and a negligible proportion of red blood cells from up to 5.3 liters of blood cardioplegia.

We have used the Pall BC-1 leukocyte filter in a pig model at a flow rate of 150–300 ml/min to maintain a leukocyte depletion of more than 90% [42]. Therefore, the filter had to be replaced after a total blood volume of 1,300 ml (fig. 1).

The fibers of polyester leukocyte filters with the adhering leukocytes may activate complement and result in subsequent leukocyte chemotaxis, thereby stimulating granulocyte migration toward ischemically damaged myocardium and potentially blunting the beneficial effects of leukopheresis [34, 43]. To avoid excessive release of free radicals, enzymes, cytokines and other chemotactic factors from the activated leukocytes the filters should be removed from blood circulation when their capacity is exhausted.

Leukocyte Depletion in Experimental Studies

In numerous experimental studies, leukocyte depletion of reperfusate blood has been shown to decrease myocardial necrosis after reperfusion of acutely ischemic myocardium [44–49].

Experimental Heart Transplantation

To minimize reperfusion injury, leukocyte-depleted blood cardioplegia was successfully used in extreme experimental models of myocardial ischemia,

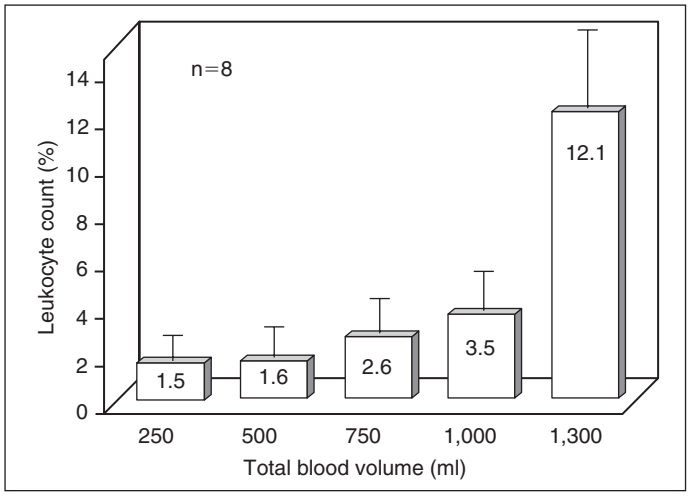


Fig. 1. Leukocyte depletion of porcine blood using the Pall BC1B filter. To maintain a leukocyte depletion of more than 90% the filter has to be replaced after a total blood volume of approximately 1,300 ml. Data are given as mean ± SD.

e.g. transplantation from non-heart-beating donors after unprotected normothermic global ischemia of 30 min [50].

We have described a technique for successful transplantation of pig hearts after 30 min of normothermic ischemia without donor pretreatment [42, 51]. In this experimental model of severe myocardial ischemia the hearts developed ischemic contracture within a few minutes after initial reperfusion with unfiltered blood cardioplegia. It was not possible to wean the hearts from CPB despite controlled reperfusion with substrate-enriched blood cardioplegia. After addition of a leukocyte blood cardioplegia filter to the reperfusion regime cardiac contractility was markedly improved and the animals could be weaned successfully.

Fukushima et al. [52] compared leukocyte-depleted blood cardioplegia versus undepleted blood cardioplegia after 24-hour preservation of canine hearts for heterotopic transplantation. Only leukocyte-depleted blood cardioplegia was able to replenish the energy-depleted myocardium, preserve coronary flow and contractile function, and to reduce lipid peroxidation.

Regional Myocardial Ischemia

In a pig model, second and third diagonal vessels were occluded for 90 min, followed by 45 min of blood cardioplegic arrest and 180 min of reperfusion on CPB [53]. In three experimental groups leukocyte filters were inserted

(a) in both CPB and blood cardioplegia circuits, (b) in CPB only, and (c) in the blood cardioplegia circuit only. Leukocyte filters appeared to be most effective compared to untreated controls when placed in the CPB circuit before cardioplegic arrest. The addition of leukocyte filters to blood cardioplegia did not significantly alter wall motion scores or the area of necrosis.

Kofsky et al. [42] tested the effect of leukocyte depletion during reperfusion in a dog model with 2 h of regional myocardial ischemia. Compared to early reperfusion with unmodified blood, leukocyte depletion resulted in a lower incidence of ventricular fibrillation, decreased coronary vascular resistance and limited histochemical damage. In contrast, improvement of regional contractility could not be observed after leukocyte filtration but only when blood cardioplegia was used. Neutrophil filtration of blood cardioplegic solutions did not further enhance the salutary effects of blood cardioplegia.

Similar results were obtained by Byrne et al. [46] after 90 min of regional myocardial ischemia. Leukocyte filtration versus reperfusion with unmodified whole blood improved the left ventricular stroke work index, mean rise of left ventricular pressure and myocardial blood flow. No other adjuncts to control reperfusion, e.g. substrate-enriched blood cardioplegia, were given in this study.

Schmidt et al. [54] investigated leukocyte-depleted blood cardioplegia in a canine model of 90 min of regional myocardial ischemia, followed by a 60-min cardioplegic arrest using continuous blood cardioplegia. They found no effect on global ventricular function or water content but the endothelial function was significantly better preserved in the leukocyte-depleted group.

In a model of severe myocardial ischemia (12 h of cardioplegic arrest in neonatal piglet hearts) Breda et al. [55] could show that reperfusion with neutrophil-depleted blood was associated with improved postischemic function, a threefold higher coronary blood flow, and preserved ultrastructure of the myocardium on electron microscopic examination compared to the control group.

Leukocyte-Depleted Blood Cardioplegia in the Clinical Arena

Heart Transplantation

Inadequate myocardial protection associated with ischemic and reperfusion injury is an important reason for early graft failure after heart transplantation. Furthermore, suboptimal early graft function is common, and high-dose inotropic support is frequently required postoperatively. Reperfusion with substrate-enriched, leukocyte-depleted blood cardioplegic solution for the first 3 min of reperfusion and leukocyte-depleted blood for an additional 7 min after prolonged hypothermic ischemia resulted in shorter duration of inotropic support

and decreased leakage of myocardial enzymes, and prevented ultrastructural injury [56, 57].

Elective and Emergency Open Heart Surgery

In patients undergoing elective open-heart surgery the use of a leukocyte-depleting filter for blood cardioplegia resulted in a significantly diminished release of creatine kinase and troponin T as compared to the control group [58].

In a randomized study, De Vecchi et al. [59] investigated the effect of leukocyte-depleted blood cardioplegia in patients with normal and depressed ventricular function undergoing coronary bypass. In patients with depressed ejection fraction, the recovery rate of plasma glutathione redox ratio (oxidized/total glutathione) was significantly faster in the leukocyte-depleted versus control group. In patients with a normal ejection fraction leukocyte depletion had no beneficial effect [59].

Sawa et al. [60] tested the effect of leukocyte filtration of blood cardioplegia for initial reperfusion after coronary bypass grafting in elective versus emergency surgery. They found significantly lower peak creatine kinase-MB (CK-MB) levels, lower myocardial malonaldehyde release and lower inotropic support at the weaning of CPB when leukocyte-depleted blood cardioplegia was used. These observations could be made only in emergency surgical procedures requiring a preoperative intra-aortic balloon pump because of developing acute myocardial infarction. In elective coronary artery bypass grafting, no benefit of leukocyte filtration was found in the clinical data.

These results are consistent with the experience that the specific contribution of neutrophils to mild or moderate myocardial damage seems less effective in contrast to the severely damaged myocardium [61, 62].

Palatinos et al. [63] investigated the effect of leukocyte-depleted blood cardioplegia in 160 patients undergoing elective coronary bypass operation. They found a significantly improved cardiac index, reduced incidence of ventricular arrhythmias during reperfusion, and decreased CK-MB and troponin I levels after 6 and 12 h.

Conversely, other studies have shown that neutrophil content in stunned myocardium is not increased [3] and that interventions to remove neutrophils have not improved function [64, 65].

Regarding the results of the existing experimental and clinical studies there is no doubt that leukocyte filtration of blood cardioplegia can attenuate reperfusion injury. To provide maximal effectiveness of leukocyte filters, the surgeon should settle the following questions:

(1) To what level is it necessary to deplete the leukocyte count to see an effect? Does the level of depletion correlate with the effects? Engler et al. [66] showed that 10% of the normal circulating granulocyte count was sufficient to

cause a significant postischemic ventricular dysfunction after only 15 min of regional ischemia. Therefore, more than 90% of leukocytes should be removed and the filters replaced when their capacity is exhausted. The use of leukocyte filters for blood cardioplegia seems to provide more advantages as compared to arterial line filters. It remains to be determined whether complete leukocyte depletion can be achieved in the systemic circulation and whether it would lead to clinical problems. Conversely, some studies revealed that incomplete depletion of leukocytes does not worsen the clinical outcome [67].

(2) How long should leukocyte depletion be maintained after starting reperfusion? Accumulation of neutrophils occurs within the first 10 min of reperfusion. Depending on the severity of tissue injury, leukocyte depletion should be maintained at least 10–20 min. In a lung model of 24-hour hypothermic preservation addition of leukocytes after 1 h of leukocyte-depleted reperfusion did not cause measurable injury, whereas addition of leukocytes at 30, 15, and 5 min caused progressive functional loss [68].

After discontinuation of mechanical leukocyte filtration Wilson et al. [44] observed a reduced tendency to accumulate during late reperfusion despite the increase in circulating leukocytes. This could be due to a reduction in myocardial chemotactic factors during initial reperfusion or to a reduction in the surface expression of neutrophil adherence receptors during early reperfusion.

Conclusions

(1) The effect of leukocyte depletion during reperfusion depends on the degree of myocardial damage. The most benefit can be expected in ischemic hearts or hearts with impaired ventricular function. But there is some evidence that leukocyte filtration also has positive effects in routine open heart surgery.

(2) Leukocyte depletion is most effective during early reperfusion and should be maintained for at least 10–20 min.

(3) The leukocyte level in the initial reperfusate should be depleted to less than 10% to avoid myocardial damage due to activated leukocytes.

(4) Leukocyte depletion alone may have a limited effect in vulnerable myocardium to attenuate reperfusion injury and should therefore be combined with substrate-enriched blood cardioplegia and controlled reperfusion conditions.

References

1 Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork A, Lucchesi BR: Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. Circulation 1983;67: 1016–1023.

2 Rossen RD, Swain JL, Michael LH, Weakley S, Giannini E, Entman NL: Selective accumulation of the first component of complement and leukocytes in ischemia canine heart muscle: A possible initiator of an extra myocardial metabolism of ischemic injury. Circ Res 1985;57:119–130.
3 Go LO, Mury CE, Richard VJ, Weischedel GR, Jennings RB, Reimer KA: Myocardial neutrophil accumulation during and after reversible or irreversible ischemic injury. Am J Physiol 1988;225: H1188–1198.
4 Metha J, Nichols W, Metha P: Neutrophils as potential participants in acute myocardial ischemia: Relevance to reperfusion. J Am Coll Cardiol 1988;11:1309–1316.
5 Weiss SJ: Tissue destruction by neutrophils. N Engl J Med 1989;320:365–376.
6 Forman MB, Virmani R, Puett DW: Mechanisms and therapy of myocardial reperfusion injury. Circulation 1990;81(suppl IV):IV69–IV78.
7 Ceriana P: Effect of myocardial ischemia-reperfusion on granulocyte elastase release. Anaesth Intensive Care 1992;20:187–190.
8 Farah B, Vuillemenot A, Lecompte T, Bara L, Pasquier C, Jebara V, Carpentier A, Fabiani JN: Myocardial neutrophil sequestration and activation related to the reperfusion of human heart during coronary artery surgery. Cardiovasc Res 1994;28:1226–1230.
9 Hansen PR: Role of neutrophils in myocardial ischemia and reperfusion. Circulation 1995; 91:1872–1885.
10 Jordan JE, Zhao ZQ, Vinten-Johansen J: The role of neutrophils in myocardial ischemia-reperfusion injury. Cardiovasc Res 1999;860–878.
11 Engler RL, Dahlgren MD, Peterson MA, Dobbs A, Schmid-Schoenbein G: Accumulation of polymorphonuclear leukocytes during 3-hour experimental myocardial ischemia. Am J Physiol 1986;251:H93–H100.
12 Harlan JM: Leukocyte-endothelial interactions. Blood 1985;65:513–525.
13 Harlan JM, Killen PD, Senecal FM, Schwartz BR, Yee EK, Taylor RF: The role of neutrophil membrane glycoprotein GP-150 in neutrophil adherence to endothelium in vitro. Blood 1985;66:167–178.
14 Babior BM: The respiratory burst of phagocytes. J Clin Invest 1984;73:599–601.
15 Ferrari R, Ceconi C, Curello S, Guarnieri C, Caldarera CM, Albertini A, Visioli O: Oxygen-mediated myocardial damage during ischemia and reperfusion: Role of the cellular defences against oxygen toxicity. J Mol Cell Cardiol 1985;17:937–945.
16 Petrone WF, English DK, Wong K, Mc Cord JM: Free radicals and inflammation: Superoxide-dependent activation of a neutrophil chemotactic factor in plasma. Proc Natl Acad Sci USA 1980; 77:1159–1163.
17 Lucchesi BR: Role of neutrophils in ischemic heart disease: Pathophysiologic role in myocardial ischemia and coronary artery reperfusion; in Mehta JL (ed): Thrombosis and platelets in myocardial ischemia. Philadelphia, Davis, 1987; pp 35–48.
18 Le Deist F, Menasche P, Kucharski C, Bel A, Piwnica A, Bloch G: Hypothermia during cardiopulmonary bypass delays but does not prevent neutrophil-endothelial cell adhesion. A clinical study. Circulation 1995;92(suppl):II354–II358.
19 Bolli R, Riana JF, Jeroudi M: Prolonged impairment of coronary vasodilation after reversible ischemia. Evidence for microvascular 'stunning'. Circ Res 1990;67:332–343.
20 Vanhaecke J, Flameng W, Borgers M, Jang IK, Van de Werf F, De Geest H: Evidence for decreased coronary flow reserve in viable postischemic myocardium. Circ Res 1990;67:1201–1210.
21 Pearl JM, Laks H, Drinkwater DC, et al: Loss of endothelium-dependent vasodilatation and nitric oxide release after myocardial protection with University of Wisconsin solution. J Thorac Cardiovasc Surg 1994;107:257–264.
22 Ge ZD, He GW: Altered endothelium-derived hyperpolarizing factor-mediated endothelial function in coronary microarteries by St Thomas' Hospital solution. J Thorac Cardiovasc Surg 1999; 118:173–180.
23 Buckberg GD: Editorial: Endothelial and myocardial stunning. J Thorac Cardiovasc Surg 2000; 120:640–641.
24 Stangl V, Rodiger O, Frank TM, Bohm M, Stangl K, Baumann G, Felix SB: Influence of polymorphonuclear leukocytes and plasma on coronary vasomotion after ischemia. Ann Thorac Surg 1999;68:442–446.

25 Olafsson B, Forman MB, Puett DW, Pou A, Cates CU, Freisinger GC, Virmani R: Reduction of reperfusion injury in the canine preparation by intracoronary adenosine: Importance of the endothelium and the no-reflow phenomenon. Circulation 1987;76:1135–1145.
26 Vinten-Johansen J, Thourani VH, Ronson RS, Jordan JE, Zhao ZY, Nakamura M, Velez D, Guyton RA: Broad-spectrum cardioprotection with adenosine. Ann Thorac Surg 1999;68: 1942–1948.
27 Berne RM: The role of adenosine in the regulation of coronary blood flow. Circ Res 1980;47: 807–813.
28 Auchampach JA, Maruyama M, Carero I, Gross GJ: Pharmacological evidence for a role of ATP-dependent potassium channels in myocardial stunning. Circulation 1992;86:311–319.
29 Hudspeth DA, Nakanishi K, Vinten-Johansen J, Zhao J-Q, McGee DS, Williams MW, Hammon JW Jr: Adenosine in blood cardioplegia prevents postischemic dysfunction in ischemically injured hearts. Ann Thorac Surg 1994;58:1637–1644.
30 Cronstein BN, Levin RI, Belanoff J, Weissmann G, Hirschhorn R: Adenosine: An endogenous inhibitor of neutrophil-mediated injury to endothelial cells. J Clin Invest 1986;78:760–770.
31 Vinten-Johansen J, Zhao ZQ, Sato H: Reduction in surgical ischemic-reperfusion injury with adenosine and nitric oxide therapy. Ann Thorac Surg 1995;60:852–857.
32 Sato H, Zhao ZQ, Jordan JE, Todd JC, Riley RD, Taft CS, Hammon JW Jr, Li P, Ma X, Vinten-Johansen J: Basal nitric oxide expresses endogenous cardioprotection during reperfusion by inhibition of neutrophil-mediated damage after surgical revascularization. J Thorac Cardiovasc Surg 1997;113:399–409.
33 Vinten-Johansen J, Buckberg GD, Okamoto F, Rosenkranz ER, Bugyi H, Leaf J: Studies of controlled reperfusion after ischemia. V. Superiority of surgical versus medical reperfusion after regional ischemia. J Thorac Cardiovasc Surg 1986;92:525–534.
34 Hammerschmidt DE, Craddock PR, Mc Cullough J, Kronenberg RS: Complement activation and pulmonary leukostasis during nylon fiber filtration leukapheresis. Blood 1978;51:721–728.
35 Hill JH, Ward PA: The phlogistic role of C3 leukotactic fragments in myocardial infarcts of rats. J Exp Med 1971;133:885–900.
36 Bagge U, Amundson B, et al: White blood cell deformability and plugging of skeletal muscle capillaries in hemorrhagic shock. Acta Physiol Scand 1980;105:159–163.
37 Engler RL, Dahlgren MD, Morris D, Peterson MA, Schmid-Schoenbein G: Role of leukocytes in the response to acute myocardial ischemia and reflow in dogs. Am J Physiol 1986;251:H314–H323.
38 Dhote-Burger P, Vuillemenot A, Lecompte T, Julia P, Pasquier C, Chardigny C, Fabiani JN: Is myocardial function influenced by neutrophil activation during ischemia and reperfusion in coronary artery surgery? Transplant Proc 1995;27:2786–2788.
39 Byrne JG, Smith WJ, Murphy MP, Couper GS, Appleyard RF, Cohn LH: Complete prevention of myocardial stunning, contracture, low-reflow, and edema after heart transplantation by blocking neutrophil adhesion molecules during reperfusion. J Thorac Cardiovasc Surg 1993;104:1589–1596.
40 Sirchia G, Rebulla P, Parravicini A, Carnelli V, Gianotti GA, Bertolini F: Leukocyte depletion of red blood cell units at the bedside by transfusion through a new filter. Transfusion 1987;27: 402–405.
41 Heggie AJ, Corder JS, Crichton PR, Hesford JW, Bingham H, Jeffries S, Hooper TL: Clinical evaluation of the new Pall leukocyte-depleting blood cardioplegia filter (BC1). Perfusion 1998; 13:17–25.
42 Martin J, Sarai K, Yoshitake M, Haberstroh J, Takahashi N, Lutter G, Beyersdorf F: Orthotopic transplantation of pig hearts harvested after 30 min of normothermic ischemia: Controlled reperfusion with blood cardioplegia containing the Na^+-H^+-exchange inhibitor HOE 642. Eur J Cardiothorac Surg 1998;14:607–614.
43 Kofsky ER, Julia PL, Buckberg GD, Quillen JE, Acar C: Studies of controlled reperfusion after ischemia. XXII. Reperfusate composition: Effects of leukocyte depletion of blood and blood cardioplegic reperfusates after acute coronary occlusion. J Thorac Cardiovasc Surg 1991;101: 350–359.
44 Westlin M, Mullane KM: Allevation of myocardial stunning by leukocyte and platelet depletion. Circulation 1989;80:1828–1836.

45 Wilson IC, Gardner TJ, DiNatale JM, Gillinov AM, Curtis WE, Cameron DE: Temporary leucocyte depletion reduces ventricular dysfunction during prolonged postischemic reperfusion. J Thorac Cardiovasc Surg 1993;106;805–810.
46 Litt MR, Jeremy RW, Weisman HF, Winkelstein JA, Becker LC: Neutrophil depletion limited to reperfusion reduces myocardial infarct size after 90 minutes of ischemia. Circulation 1989;80: 1816–1827.
47 Byrne JG, Appleyard RF, Lee CC, et al: Controlled reperfusion of the regionally ischemic myocardium with leucocyte-depleted blood reduces stunning, the no-reflow phenomenon, and infarct size. J Thorac Cardiovasc Surg 1992;103:66–72.
48 Sawa Y, Nakano S, Shimazaki Y, Nishimura M, Kuratani T, Matsuda H: Myocardial protective effect and its mechanism of leukocyte-depleted reperfusion in neonatal rabbit hearts. Ann Thorac Surg 1994;58:1386–1391.
49 Kawata H, Sawatari I, Mayer E: Evidence for the role of neutrophils in reperfusion injury after cold cardioplegic ischemia in neonatal lambs. J Thorac Cardiovasc Surg 1992;103:908–918.
50 Gundry SR, Fukushima N, Eke CC, Hill AC, Zuppan C, Bailey LL: Successful survival of primates receiving transplantation with 'dead', nonbeating donor hearts. J Thorac Cardiovasc Surg 1995;109:1097–1102.
51 Martin J, Sarai K, Yoshitake M, Haberstroh J, Takahashi N, Lutter G, Beyersdorf F: Successful orthotopic pig heart transplantation from non-heart-beating donors. J Heart Lung Transplant 1999;18:597–606.
52 Fukushima N, Shirakura R, Nakata S, Kaneko M, Miyagawa S, Naka Y, Chang JC, Matsumiya G, Nakano S, Matsuda H: Study of efficacies of leukocyte-depleted terminal blood cardioplegia in 24-hour preserved hearts. Ann Thorac Surg 1994;58:1651–1656.
53 Lazar HL, Zhang Xi, Takafumi H, Treanor P, Rivers S, Bernard S, Shemin RJ: Role of leukocyte depletion during cardiopulmonary bypass and cardioplegic arrest. Ann Thorac Surg 1995;60: 1745–1748.
54 Schmidt FE Jr, MacDonald MJ, Murphy CO, Brown WM, Gott JP, Guyton RA: Leukocyte depletion of blood cardioplegia attenuates reperfusion injury. Ann Thorac Surg 1996;62:1691–1696.
55 Breda MA, Drinkwater DC, Laks H, et al: Prevention of reperfusion injury in the neonatal heart with leucocyte-depleted blood. J Thorac Cardiovasc Surg 1989;96:654–665.
56 Pearl JM, Drinkwater DC, Laks H, Stein DG, Capouya ER, Bhuta S: Leukocyte-depleted reperfusion of transplanted human hearts prevents ultrastructural evidence of reperfusion injury. J Surg Res 1992;52:298–308.
57 Pearl J, Drinkwater DC, Laks H, Capouya ER, Gates RN: Leukocyte-depleted reperfusion of transplanted human hearts: A randomized, double-blind clinical trial. J Heart Lung Transplant 1992;11:1082–1092.
58 Suzuki I, Ogoshi N, Chiba M, Komatsu T, Moizumi Y: Clinical evaluation of a leukocyte-depleting blood cardioplegia filter (BC1B) for elective open-heart surgery. Perfusion 1998;13: 205–210.
59 De Vecchi E, Paroni R, Pala MG, Di Credica G, Agape V, Gobbi C, Bonini PA, Paolini G, Grossi A: Role of leucocytes in free radical production during myocardial revascularization. Heart 1997;77:449–455.
60 Sawa Y, Matsuda H, Shimazaki Y, Kaneko M, Nishimura M, Amemiya A, Sakai K, Nakano S: Evaluation of leukocyte-depleted terminal blood cardioplegic solution in patients undergoing elective and emergency coronary artery bypass grafting. J Thorac Cardiovasc Surg 1994;108: 1125–1131.
61 Reimer KA, Murry CE, Richard VJ: The role of neutrophils and free radicals in the ischemic reperfused heart: Why the confusion and controversy. J Mol Cell Cardiol 1989;21:1225–1239.
62 O'Neill PG, Charlat ML, Michael LH, Roberts R, Bolli R: Influence of neutrophil depletion of myocardial function and flow after reversible ischemia. Am J Physiol 1989;256:H341–351.
63 Palatinos GM, Balentie B, Astras G, Prapas S, Triantafillou C, Panagiotou M, Papadakis EG, Stratigi P, Vassili M, Lidoriki A, Dinopoulos T: Leukocyte fitration reduces myocardial reperfusion morbidity. 36th Annual Meeting of the Society of Thoracic Surgeons, Fort Lauderdale, 2000.

64 Jeremy RW, Becker LC: Neutrophil depletion does not prevent myocardial dysfunction after brief coronary occlusion. J Am Coll Cardiol 1989;13:1155–1163.
65 Carlson RE, Schott RJ, Buda AJ: Neutrophil depletion fails to modify myocardial no reflow and functional recovery after coronary reperfusion. J Am Coll Cardiol 1989;14:1803–1813.
66 Engler RL: Free radical and granulocyte-mediated injury during myocardial ischemia and reperfusion. Am J Cardiol 1989;63:19E–23E.
67 Paianzo DA, Manley NJ, Montesano RM, Yeisley GL, Gordon D: Clinical evaluation of the LeukoGuard (LG6) arterial line filter for routine open heart surgery. Perfusion 1993;8:489–496.
68 Hall TS, Breda MA, Baumgartner WA, Borkon AM, Brawn J, Hutchins GM, Reitz BA: The role of leukocyte depletion in reducing injury to the lung after hypothermic ischemia. Curr Surg 1987; 44:137–139.

Juergen Martin, MD, Division of Cardiovascular Surgery,
Albert Ludwig University, Hugstetter Strasse 55, D–79106 Freiburg (Germany)
Tel. +49 761 270 2818, Fax +49 761 270 2550, E-mail Martin@ch11.ukl.uni-freiburg.de

Matheis G, Moritz A, Scholz M (eds): Leukocyte Depletion in Cardiac Surgery and Cardiology.
Karger, Basel, 2002, pp 147–153

Future Perspectives to Limit the Pathogenicity of Leukocytes in Cardiac Surgery and Cardiology

Markus Berg, Martin Scholz, Georg Matheis

Department of Thoracic and Cardiovascular Surgery, J.W. Goethe University, Frankfurt, Germany

The consensus of this book may be expressed as follows: leukocyte filtration has been shown to have beneficial effects on the outcome of cardiac surgery or cardiologic interventions, but strategic modifications are necessary to make this tool more powerful in preventing leukocyte-mediated disease. Therefore, this chapter summarizes the efforts that have been discussed in this book to optimize leukocyte filtration and the prevention of leukocyte-mediated disease in the future. The proposed improvements, alone or in combination with other strategies, may be of extensive benefit to the patients. At the end of this contribution, we will propose a novel filter type, that not only depletes leukocytes, but also has bioactive properties to limit the pathogenic effects of soluble factors released by cells entrapped in the filter mesh.

Strategic Systemic Filtration

Cardiac surgery with cardiopulmonary bypass (CPB) is a highly complex pathologic situation that consists of leukocyte activation by artificial surfaces, endothelial activation, oxidative stress. It is conceivable that the prevention of tissue injury resulting from these pathomechanisms cannot be achieved by solely focussing on one pathological aspect from this system. It is therefore suggested to modify more than one pathogenic factor in order to improve clinical outcome. Recently, it has been reported that heparinized CPB circuits alone did not unanimously result in improved clinical outcome and/or reduced levels

of inflammatory parameters. However, heparinized circuits in combination with strategic leukocyte filtration are superior to each modification alone [1, 2]. Currently the optimum timing of leukocyte depletion during CPB/reperfusion is studied. One rationale is to use an arterial blood filter as a standard if required by law or deemed appropriate for other reasons, and to use a leukocyte depletion filter in parallel. For certain time periods blood flow can be switched from the standard filter to the leukocyte filter.

In addition, leukocytes entrapped within the filter probably secrete soluble pathogenic factors, such as complement factors, enzymes, cytokines. These factors may circulate in the blood regardless of leukocyte depletion efficacy. Indeed, in clinical studies with leukocyte depletion filters significant trans-filter reductions in the concentration of various soluble factors were absent [unpubl. obs.]. Moreover, leukocyte functions such as phagocytosis, and oxidative burst do not appear significantly impaired. These results support further modifications of leukocyte depletion strategies.

Leukocyte Depletion in the Venous Line

The recent study by Gu et al. [3] on the feasibility of low flow filtration in the venous line focusses on an area worth exploring. In this study leukocyte filtration reduced neutrophil counts and the potent chemoattractant IL-8 [3]. A more recent study supported the feasibility of this procedural modification [4]. The authors showed significant removal of activated leukocytes and further suggested the absence of activation of granulocytes by filtration because β-glucuronidase concentration did not increase after filtration.

Leukocyte Filtration of Blood Cardioplegia

Despite numerous modifications of CPB including heparin coating [5], protein coating [6], surface modification [7], pulsatile CPB [8], myocardial damage due to reperfusion injury after release of the aortic cross clamp remains largely unaffected by these interventions, and has been advanced mainly by the introduction of blood cardioplegic regimens [9]. The pathology of reperfusion injury may be largely based on oxidative stress and leukocyte-mediated disturbance of endothelial integrity. A considerable body of evidence exists regarding myocardial protection by leukocyte filters in the blood cardioplegia line [10–12]. The (repetitive) reperfusion of the coronary circulation with blood cardioplegia is a highly pathogenic situation in that it causes repeated ischemia/reperfusion injury as described above. The coronary circulation cannot sufficiently

be addressed by arterial inline leukocyte filters during aortic cross clamping unless the blood required for cardioplegia is withdrawn behind the arterial filter. Specific leukocyte-depleting blood cardioplegia filters (BC-1, Pall, Asahi 80) have been developed. They differ in priming volume (Asahi 80 ml, Pall 220 ml) and have been shown to be safe and efficient in terms of low resistance, leukocyte reduction (70%), and low platelet depletion rate [13]. It has been demonstrated that leukocyte depletion of blood cardioplegia reduces the magnitude of myocardial damage and improves cardiac function as determined by stroke work index, necrosis, and endothelial dysfunction with edema. More specifically, Sawa et al. [14] found reduced creatine kinase-MB (CK-MB) levels, reduced parameters of oxidative damage and lower requirement for inotropic support. Similarly, by using cold blood cardioplegia, Ichihara et al. [15] have shown lower values of lipid peroxide, elastase and CK-MB in the leukocyte depletion group. Recently, it has been confirmed by Hayashi et al. [16] and Roth et al. [17] that myocardial cell injury can be significantly affected by the use of leukocyte-depleted blood cardioplegia as a result of limited oxidative damage (e.g. lower MDA levels in coronary sinus blood).

Leukocyte Filtration of the Suction Blood

Leukocyte filtration of residual cardiotomy suction blood from the heart-lung machine should be considered in cardiac surgery with CPB. Untreated suction blood contains significant amounts of debris, soluble factors such as proinflammatory cytokines and activated leukocytes. However, transfusion leukocyte filters used in this context tend to occlude rapidly. Gu et al. [18] investigated the residual blood from the heart-lung machine, and found that leukocyte depletion of this residual pump blood improves postoperative pulmonary gas exchange functions.

Pharmacology to Limit the Pathogenic Effects of Leukocytes

Multiple unsuccessful efforts have been made in the past to pharmacologically limit leukocyte pathogenicity in various clinical situations. A major focus of current cardiovascular research is to understand and to prevent the cerebral disorders observed after cardiac surgery. In the section ‘General Considerations’, the pathomechanisms of leukocyte-mediated disturbance of the blood-brain barrier as a consequence of cardiac surgery with CPB are proposed to be related to the impairment of tight junction molecules. In a clinical study with the Na^+/H^+ exchange system inhibitor cariporide (HOE642) a significant reduction of the astroglial marker S100B was found in the serum after cardiac surgery.

It is therefore conceivable that HOE642 protects the microvascular endothelium [19]. Indeed, HOE642 experimentally stabilized tight junctions in cell cultures in the presence of phorbol myristate acetate, which simulates stress-associated protein kinase C activation and disrupts intercellular junctions via intracellular signaling. Further pharmacological studies ought to be done to define pharmacologic strategies that stabilize the interendothelial cell contacts alone or in combination with other interventions, such as inhibition of leukocyte-endothelial cell interactions and/or leukocyte depletion.

Biotechnology to Limit Leukocyte Pathogenicity

Lipid Filtration and Embolex

Currently, filtration in CPB is in the process of extremely dynamic development. Standard arterial inline filters in conjunction with membrane oxygenators have been challenged because of insufficient evidence of a clinical benefit. Various other filtration technologies for CPB and cardiac surgery are currently evolving. Intra-aortic particle filters (EmbolX) address emboli originating from the left heart and the ascending aorta, and the need for lipid filters to abolish the neuropathogenicity of lipids retransfused with cardiotomy suction blood. On the other hand OP-CAB procedures with minimized extracorporeal circuits without a reservoir and therefore without the use of cardiotomy suction (Jostra MECC system) are currently evolving. They may limit leukocyte pathogenicity due to the absence of cardiotomy suction and therefore assist current and evolving leukocyte filtration strategies.

Bioactive Filter

As mentioned above, leukocyte entrapment may result in adhesion-mediated release of cytokines and enzymes both contributing to endothelial activation and/or dysfunction. Elastase-proteinase inhibitor complex levels were found to be rather augmented in patient plasma after leukocyte filtration. However, the functional activity of elastase revealed no significant difference between the filter group and the controls, indicating that the capacity of plasma to buffer free elastase was still sufficient at least in these clinical settings. Not only enzymes but also proinflammatory cytokines, such as IL-1 and TNF-α or reactive oxygen species may be secreted. As we learned from our current studies, leukocytes can be stimulated to a normal degree after passing the filter, and thus leukocyte depletion may have only a short-term effect. Although leukocyte counts may be transiently reduced by leukocyte filtration, the residual neutrophils may still be activated and elicit endothelial and tissue damage. There may be no leukocyte count gradient across the arterial line leukocyte filter

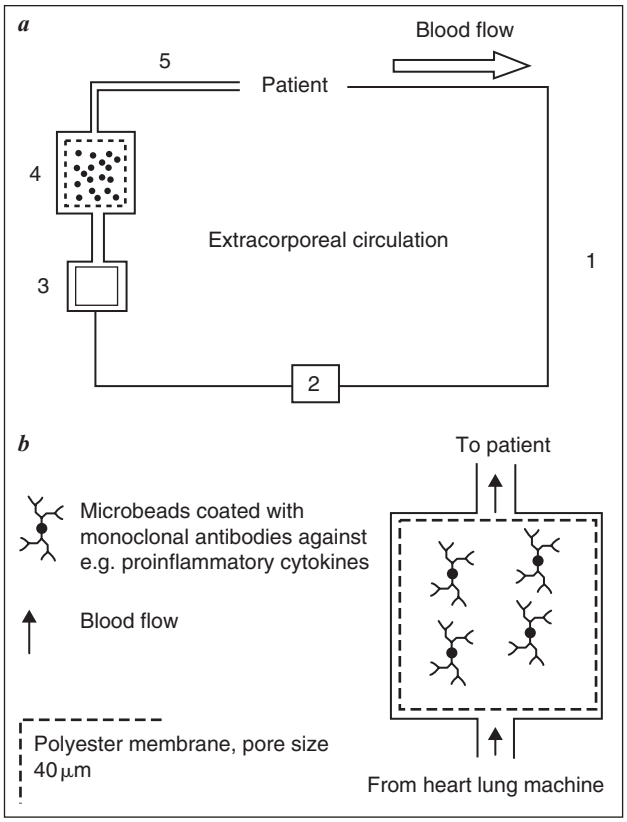


Fig. 1. a Schematic overview of the extracorporeal circuit including the leukocyte depletion filter and the bioactive filter. 1 = venous line; 2 = pump system; 3 = leukocyte filter; 4 = bioactive filter; 5 = arterial line. ***b*** Detail scheme of the example for a bioactive filter with microbeads coated with specific monoclonal antibodies, e.g. against proinflammatory cytokines.

because leukocytes can be recruited within minutes from the bone marrow and/or by demargination, as described in detail by Krishnadasan et al. [20] in this book. This rise in neutrophil counts during CPB entails clinical complications and potential limitations of the leukocyte-depleting filter.

For these reasons a bioactive and a more 'intelligent' leukocyte depletion filter is needed. For example, a polyester membrane with 40-μm pore size as it is utilized for the standard leukocyte depletion filters or antibody-coated microbeads (fig. 1) could be used for additional antibody binding against major immunodominant cytokines or chemokines such as IL-1, IL-6, IL-8, TNF-α. In

addition, the induction of apoptosis might be a possible tool to inactivate the entrapped neutrophils. Experiments are currently under way to prove the efficacy of Fas/FasL-dependent apoptosis. Polyester-bound FasL has been shown to induce apoptosis in activated Fas+ neutrophils and in Fas+ but not Fas– Jurkat cells (activated T cell line). Moreover, polymorphonuclear elastase activity was shown to be reduced in these experiments. Nevertheless, it has to be taken into account that the Fas/FasL pathway also may induce proinflammatory pathways in the neutrophil, e.g. via caspase 1. However, the concept of the proposed bioactive filter might be the beginning of a new era of leukocyte depletion in cardiac surgery and cardiology.

References

1 Matheis G, Scholz M, Gerber J, Abdel-Rahman U, Wimmer-Greinecker G, Moritz A: Leukocyte filtration in the early reperfusion phase on cardiopulmonary bypass reduces myocardial injury. Perfusion, in press.
2 Matheis G, Scholz M, Simon A, Henrich D, Wimmer-Greinecker G, Moritz A: Timing of leukocyte filtration during cardiopulmonary bypass. Perfusion, in press.
3 Gu YJ, de Vries AJ, Vos P, Boonstra PW, van Oeveren W: Leukocyte depletion during cardiac operation: A new approach through the venous bypass circuit. Ann Thorac Surg 1999;67: 604–609.
4 Smit JJ, de Vries AJ, Gu YJ, van Oeveren W: Filtration of activated granulocytes during cardiopulmonary bypass surgery: A morphologic and immunologic study to characterize the trapped leukocytes. J Lab Clin Med 2000;135:238–246.
5 Gott JP, Cooper WA, Schmidt FE Jr, Brown WM 3rd, Wright CE, Merlino JD, Fortenberry JD, Clark WS, Guyton RA: Modifying risk for extracorporeal circulation: Trial of four anti-inflammatory strategies. Ann Thorac Surg 1998;66:747–753.
6 Wimmer-Greinecker G, Matheis G, Martens S, Oremek G, Abdel-Rahman U, Moritz A: Synthetic protein treated versus heparin coated cardiopulmonary bypass surfaces: Similar clinical results and minor biochemical differences. Eur J Cardiothorac Surg 1999;16:211–217.
7 Gu YJ, Boonstra PW, Rijnsburger AA, Haan J, van Oeveren W: Cardiopulmonary bypass circuit treated with surface-modifying additives: A clinical evaluation of blood compatibility. Ann Thorac Surg 1998;65:1342–7134.
8 Taylor KM: Pulsatile reperfusion: the effects on coronary blood flow and myocardial ischemic injury. J Thorac Cardiovasc Surg 1983;85:941–942.
9 Buckberg GD: Oxygenated cardioplegia: blood is a many splendored thing. Ann Thorac Surg 1990;50:175–177.
10 Suzuki I, Ogoshi N, Chiba M, Komatsu T, Moizumi Y: Clinical evaluation of a leucocyte-depleting blood cardioplegia filter (BC1B) for elective open-heart surgery. Perfusion 1998;13:205–210.
11 Kofsky ER, Julia PL, Buckberg GD, Quillen JE, Acar C: Studies of controlled reperfusion after ischemia. XXII. Reperfusate composition: Effects of leukocyte depletion of blood and blood cardioplegic reperfusates after acute coronary occlusion. J Thorac Cardiovasc Surg 1991;101: 350–359.
12 Schmidt FE Jr, MacDonald MJ, Murphy CO, Brown WM 3rd, Gott JP, Guyton RA: Leukocyte depletion of blood cardioplegia attenuates reperfusion injury. Ann Thorac Surg 1996;62: 1691–1696.
13 Heggie AJ, Corder JS, Crichton PR, Hesford JW, Bingham H, Jeffries S, Hooper TL: Clinical evaluation of the new Pall leucocyte-depleting blood cardioplegia filter (BC1). Perfusion 1998;13: 17–25.

14 Sawa Y, Schaper J, Roth M, Nagasawa K, Ballagi G, Bleese N, Schaper W: Platelet-activating factor plays an important role in reperfusion injury in myocardium. Efficacy of platelet-activating factor receptor antagonist (CV-3988) as compared with leukocyte-depleted reperfusion. J Thorac Cardiovasc Surg 1994;108:953–959.
15 Ichihara T, Yasuura K, Maseki T, Matsuura A, Miyahara K, Ito T, Kato S, Mizuno S, Tamaki S, Seki A, et al: The effects of using a leukocyte removal filter during cold blood cardioplegia. Surg Today 1994;24:966–972.
16 Hayashi Y, Sawa Y, Nishimura M, Ichikawa H, Kagisaki K, Ohtake S, Matsuda H: Clinical evaluation of leukocyte-depleted blood cardioplegia for pediatric open heart operation. Ann Thorac Surg 2000;69:1914–1919.
17 Roth M, Kraus B, Scheffold T, Reuthebuch O, Klovekorn WP, Bauer EP: The effect of leukocyte-depleted blood cardioplegia in patients with severe left ventricular dysfunction: A randomized, double-blind study. J Thorac Cardiovasc Surg 2000;120:642–650.
18 Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W: Clinical performance of a high efficacy rapid flow leukocyte removal filter for leukocyte depletion of heparinized cardiopulmonary bypass perfusate. Perfusion 1995;10:425–430.
19 Matheis G, Scholz M, Wimmer-Greinecker G, Moritz A: Cariporide (HOE642) limits S100b release during cardiac surgery. Submitted for publication, 2001.
20 Krishnadasan B, Griscavage-Ennis J, Aldea GS, Verrier ED: Reperfusion injury during cardiopulmonary bypass; in Matheis G, Moritz A, Scholz M: Leukocyte Depletion in Cardiac Surgery and Cardiology. Basel, Karger, 2002, pp 54–77.

PD Dr. M. Scholz, Department of Thoracic and Cardiovascular Surgery,
J.W. Goethe University, Theodor-Stern-Kai 7, D–60590 Frankurt am Main (Germany)
Tel. +49 69 6301 6526, Fax +49 69 6301 7108, E-Mail m.scholz@em.uni-frankfurt.de

Author Index

Subject Index