

critical care reviews

Anemia, Allogenic Blood Transfusion, and Immunomodulation in the Critically Ill*

Murugan Raghavan, MD; and Paul E. Marik, MD, FCCP

Anemia and allogenic RBC transfusions are exceedingly common among critically ill patients. Multiple pathologic mechanisms contribute to the genesis of anemia in these patients. Emerging risks associated with allogenic RBC transfusions including the transmission of newer infectious agents and immune modulation predisposing the patient to infections requires reevaluation of current transfusion strategies. Recent data have suggested that a restrictive transfusion practice is associated with reduced morbidity and mortality during critical illness, with the possible exception of acute coronary syndromes. In this article, we review the immune-modulatory role of allogenic RBC transfusions in critically ill patients. (CHEST 2005; 127:295–307)

Key words: blood transfusion; critically ill; ICU; immune modulation; infections; microchimerism; nosocomial infection

Abbreviations: APC = antigen-presenting cell; CABG = coronary artery bypass graft; CI = confidence interval; CJD = Creutzfeldt-Jakob disease; CMV = cytomegalovirus; EPO = erythropoietin; GVHD = graft-vs-host disease; HLA = human leukocyte antigen; IL = interleukin; MHC = major histocompatibility complex; SEN-V = SEN virus; TAGVHD = transfusion-associated graft-vs-host disease; Th = T helper; TNF = tumor necrosis factor; TRALI = transfusion-related lung injury; TRIM = transfusion-induced immunomodulation; TTV = TT virus

In recent years, blood transfusion requirements have been increasing due to the increasing burden of chronic disease in an aging population, improvement in life-support technology, increasing severity of illness in patients treated in the ICU, and other blood-intensive surgical procedures.^{1,2} On the other hand, there is a trend toward decreasing blood donation and increasing cost due to the requirement for rigorous screening for transmittable infectious agents. In the United States alone, nearly 15 million U of blood are donated and 14 million U are transfused annually.² On average, 16% of patients in medical ICUs and 27% of those in surgical ICUs receive transfusions every day in the United States.³

In one series,⁴ 85% of patients with an ICU length of stay of > 1 week received at least 1 U of blood, with these patients receiving, on average, 9.5 U during their ICU stay. For decades, blood donation and transfusion were considered to be a life-saving strategy, and an arbitrary threshold of 10 g/dL was used as a transfusion trigger in critically ill patients.⁵ However, it has become evident that blood transfusion has immunomodulating effects that may increase the risk of nosocomial infections and cancer recurrence, and the possible development of autoimmune diseases later in life.^{6–10} Furthermore, the risk of “newer” transfusion-transmitted diseases has become recognized. Consequently, the safety of blood transfusions has been questioned and has led to a reevaluation of our blood transfusion practice.

*From the Department of Critical Care Medicine (Dr. Raghavan), University of Pittsburgh Medical Center, Pittsburgh; and Division of Pulmonary and Critical Care Medicine (Dr. Marik), Thomas Jefferson University, Philadelphia, PA. Manuscript received February 20, 2004; revision accepted August 12, 2004.

Reproduction of this article is prohibited without written permission from the American College of Chest Physicians (e-mail: permissions@chestnet.org).

Correspondence to: Paul Marik, MD, FCCP, Chief, Pulmonary and Critical Care Medicine, 1015 Chestnut St, Suite M100, Philadelphia, PA 19107; e-mail: paul.marik@jefferson.edu

ANEMIA AND CRITICAL ILLNESS

“Anemia of critical illness” is a common problem in the ICU. More than 90% of critically ill patients have subnormal hemoglobin levels by the third day of ICU admission.¹¹ In one series,¹² the mean hemoglobin level of patients admitted to the ICU was

11.3 g/dL, with 29% having a hemoglobin level of < 10 g/dL. Although anemia often results in extensive allogenic RBC transfusions among critically ill patients, there are insufficient data in the literature to support this widespread practice.

The etiology of anemia of critical illness is multifactorial and complex. Repeated phlebotomy procedures, GI blood loss, and other surgical procedures contribute significantly to the development of anemia.^{13,14} Critically ill patients lose approximately 25 to 40 mL blood daily through phlebotomy, and patients with indwelling arterial catheters lose approximately 900 mL blood during their ICU stay.^{9,15,16} Other important contributing factors that exacerbate anemia in critically ill patients include coagulopathies, pathogen-associated hemolysis, hypoadrenalism, and nutritional deficiencies.^{17–19}

RBC production in critically ill patients is often abnormal, and is involved in the development and maintenance of anemia. The pathophysiology of this anemia is complex, and includes the decreased production of erythropoietin (EPO), impaired bone marrow response to EPO, and reduced RBC survival.¹⁶ Critically ill patients have inappropriately low EPO concentrations, irrespective of the presence of acute renal failure.^{20–24} The suppression of EPO production by EPO gene inhibition²⁵ and EPO resistance are mediated by a variety of inflammatory mediators.²⁶ Interleukin (IL)-1, and tumor necrosis factor (TNF)- α have been shown to inhibit EPO production.²⁷ Furthermore, IL-1, IL-6, and TNF- α suppress erythropoiesis by direct inhibitory effects on bone marrow RBC production, while these effects can be reversed by exogenous EPO administration.²⁸

Decreased RBC synthesis and consequent anemia are also common during sepsis syndromes. Many ICU patients have low serum iron levels, total iron binding capacity, and elevated serum ferritin concentrations, suggesting the presence of “anemia of inflammation.” Bacteria require iron for their growth, and several studies^{29,30} have shown a link between iron and infection. It is therefore conceivable that the human host down-regulates iron metabolism and EPO synthesis as a component of nonspecific immunity during critical illness and sepsis. In addition, during sepsis low serum iron levels may also protect the host against iron-catalyzed oxidant cell damage.³¹ As RBCs also require iron for growth and maturation, anemia during sepsis may represent an adaptive mechanism by the host to starve the pathogen of iron. Thus, anemia of critical illness may also be viewed as “anemia of immune activation” and may have evolved as a protective mechanism against foreign antigens.

The most important physiologic consequence of

anemia is a reduction in the oxygen-carrying capacity of blood. These changes are accompanied by increased cardiac output, a shift of the oxyhemoglobin dissociation curve, and increased oxygen extraction. RBC transfusions in the past have been routinely employed to augment tissue oxygen delivery. Although RBC transfusions increase systemic oxygen delivery, the immediate effectiveness of stored RBC transfusions to augment tissue oxygen uptake has been questioned in several studies.^{7,32,33} Furthermore, RBC transfusion has been associated with a higher incidence of postoperative infections and nosocomial ICU infections, and poorer outcome in critically ill patients.^{34–37}

TRENDS IN TRANSFUSION PRACTICE AMONG THE CRITICALLY ILL

In a recent, large, multicentered observational study in the United States, Corwin et al³⁸ studied transfusion practices in 4,892 patients across 284 ICUs. Approximately 70% of patients who were admitted to the ICU had a baseline hemoglobin concentration of < 12 g/dL, and 44% of these patients received RBC transfusions. The mean (\pm SD) pretransfusion hemoglobin level was 8.6 ± 1.7 g/dL. The mortality rate was 10% for patients without transfusion, increasing to 25% for patients with ≥ 6 U transfused. Low hemoglobin levels were a common trigger for transfusion in approximately 90% of the patients, and the mean age of the RBCs transfused was 3 weeks; however, > 25% of transfused RBCs were > 1 month old.³⁸

In an earlier, large, multicentered, Canadian prospective trial published in 1999, Hebert et al³⁹ demonstrated that maintaining a hemoglobin level in the range of 7 to 9 g/dL was superior to a hemoglobin level of 10 g/dL, thereby raising questions regarding the validity of the historical assumption that RBC transfusions are beneficial for critically ill patients. The investigators enrolled 838 patients from 25 centers over a period of 3 years. Only normovolemic, anemic (plasma hemoglobin concentration, < 9 g/dL) patients who were expected to stay in the ICU for > 24 h were included in the study. Important exclusion criteria were evidence of active bleeding (*ie*, > 3 U transfused over 24 h), chronic anemia (plasma hemoglobin concentration < 9 g/dL in the preceding month), and cardiac surgery. The enlisted patients were randomized to receive either a restrictive transfusion strategy (hemoglobin concentration transfusion trigger, 7 g/dL; maintenance hemoglobin concentration range, 7 to 9 g/dL) or a liberal transfusion strategy (hemoglobin concentration transfusion trigger, 10 g/dL; maintenance hemoglobin con-

centration transfusion range, 10 to 12 g/dL). On average, a total of 2.6 U blood was administered to patients who were randomized to receive the restrictive strategy compared with 5.6 U for patients randomized to receive the liberal strategy. There was a nonsignificant trend toward decreased 30-day and 60-day all-cause mortality rates, and a lower adjusted multiple organ dysfunction score in favor of patients who were in the restrictive strategy group. There were significantly fewer cardiac complications, including acute myocardial infarction and pulmonary edema, observed in patients in the restrictive strategy group.

The investigators recommended a restrictive strategy as the best practice for most patients, including those with cardiovascular disease, but with the possible exception of critically ill patients with ongoing coronary ischemia. The appropriateness of a nonrestrictive transfusion approach for patients with ongoing coronary ischemia was supported by the publication of a later subgroup analysis⁴⁰ that suggested that patients with severe cardiac disease who had been randomized to the restrictive strategy group had a nonsignificant increase in the 30-day all-cause mortality rate. Unfortunately, the investigators excluded patients with chronic anemia and those undergoing cardiac surgery, and so it remains difficult to recommend a transfusion strategy for either of these groups on the basis of this study. Despite the study by Hebert et al,³⁹ Corwin et al³⁸ found that the transfusion practice in response to anemia has changed little in the United States in recent years. Since the study by Corwin et al³⁸ was initiated in early 2001 (within 2 years of the study by Hebert et al³⁹), it is conceivable that by this time the universal implementation of a restricted transfusion strategy may not have occurred in all US ICUs.

Several studies^{8,9,38,41} have suggested that routine blood transfusions increase morbidity, mortality, and length of hospital stay in critically ill patients. In the study by Corwin et al,³⁸ patients receiving transfusions had more complications, including fever, fluid overload and hypotension, sepsis, thromboembolism, and ARDS. The number of units of blood transfused was independently associated with longer ICU and hospital length of stay and increased mortality.³⁸ However, this study included a heterogeneous group of critically ill patients and did not take into consideration the specific clinical scenarios during which patients received transfusions. Furthermore, this study does not answer the question of what is the appropriate pretransfusion hemoglobin level. Similar results were confirmed by Vincent et al⁹ in an earlier large, epidemiologic, observational study conducted among 146 European ICUs involving 3,534 patients. A total of 42% of patients received transfusions with an average pretransfusion hemoglobin level of 8.4

g/dL. Patients receiving transfusions had an average length of ICU stay of 7.2 days compared with 2.6 days for patients not receiving transfusions. Both the ICU and overall mortality rates (ICU mortality rate, 18.5% vs 10.1%, respectively; overall mortality rate, 29% vs 14.9%, respectively) were significantly higher for patients receiving transfusions than for patients not receiving transfusions. Patients receiving transfusions had higher rates of organ dysfunction and mortality for every hemoglobin level when compared to patients not receiving transfusions. Using propensity scores, the authors concluded that the associated risk of death was 33% for patients receiving transfusions compared with patients not receiving transfusions. However, the study had an observational design with no control for interventions, and included a wide variety of medical, surgical, and trauma patients, thereby confounding the interpretation of the results.⁹

RBC STORAGE AND PHYSIOLOGIC ALTERATIONS

RBCs undergo various morphologic and functional changes with storage, thereby mediating some of the adverse effects associated with allogenic transfusions in critically ill patients. RBCs stored for > 15 days have a decreased ability to deform and unload oxygen in the microcirculation.⁷ Complete depletion of 2,3-diphosphoglycerate concentrations occur after 2 weeks of storage, thereby reducing the ability of transfused RBCs to offload oxygen by > 50%.^{7,42} RBC adenosine triphosphate levels have been shown to decrease following storage, resulting in a change in RBC shape from discoid to spherocytic, a loss of membrane lipid, and a decrease in cellular deformability.^{43,44} This causes capillary sludging and obstruction, thereby predisposing the patient to tissue ischemia and decreased oxygen delivery.⁴⁵ The increased adhesion of nonleukodepleted stored RBCs to endothelial cells also has been demonstrated. RBC adhesion increases with the duration of storage, and prestorage leukoreduction eliminates such storage-related adhesion.⁴⁶ Therefore, the transfusion of adhesive RBCs may further compromise tissue blood flow, leading to impaired perfusion and organ dysfunction in critically ill patients.⁴⁷ The loss of endogenous RBC antioxidants occurs during the storage of blood. This increases oxidative injury of the cytoskeleton proteins and membrane phospholipids, and results in the conversion of hemoglobin to methemoglobin, which is incapable of binding oxygen.^{48,49} The resultant tissue ischemia predisposes critically ill patients to an increased risk of infections and organ dysfunction.

WBCs are present in all cellular blood components that are prepared by standard techniques, and

many studies have indicated that leukocyte contamination of erythrocyte or platelet preparations can cause a wide range of physiologic and immunologic dysfunction in recipients.^{10,50–55} The accumulation of various soluble bioactive substances occurs during storage, and includes histamine, lipids, cytokines, fragments of cellular membranes, soluble human leukocyte antigen (HLA) class I antigens, many of which are WBC-derived and play an important role in transfusion-induced immunomodulation (TRIM). Stored RBCs harbor potent proinflammatory cytokines such as IL-1, IL-6, IL-8, bactericidal permeability-increasing protein, and TNF.^{56,57} The transfusion of stored RBCs has been shown to trigger neutrophil activation, and the release of IL-8 and secretory phospholipase A₂, thereby predisposing the patient to systemic inflammatory response syndrome.^{58,59} The WBC contamination of stored RBC concentrates also has been shown to have a direct deleterious effect on RBC integrity. Increased hemolysis, microvesiculation, and potassium leakage occurs in RBCs in stored blood with an increasing amount of WBC contamination.⁶⁰ WBC apoptosis with a resultant release of toxic oxygen radicals and WBC-associated enzymes during RBC storage and transfusion have been implicated in some of these adverse effects.⁶¹ Arginase release from stored RBCs has been implicated in transfusion-associated immunosuppression. Arginine is degraded by arginase, an enzyme that is abundantly present in RBCs. While arginine stimulates lymphocyte function, arginase impairs it. Therefore, arginase leakage from stored RBCs may be an important mediator of immunosuppression that is associated with allogenic blood transfusions.⁶²

In the United States, approximately 20% of all RBCs transfused are ≥ 28 days old, and the RBC storage duration has been identified as a potential cause for the increased morbidity and mortality that has been observed with blood transfusions in several studies.^{36,37,63,64} Clinical outcomes associated with increased storage duration include increased length of stay in the hospital/ICU, multiple organ system failure, increased infections, and impaired tissue oxygen utilization. Martin et al,⁶³ in one of the earliest retrospective analyses of 698 patients, described a relationship between the transfusion of non-leukocyte-reduced RBCs that had been stored for > 14 days and the associated increased length of stay in the ICU ($p < 0.0001$). Length of stay was significantly associated with the aging of RBCs ($p = 0.003$), the total number of units transfused ($p = 0.004$), and the median storage duration ($p = 0.02$). Furthermore, when transfused patients were analyzed separately from nontransfused patients, only RBC storage for > 14 days was independently predictive of length of stay ($p < 0.0001$).⁶³

Similarly, Purdy et al³⁶ described a positive correlation between mortality in patients with severe sepsis and the age of the non-leukocyte-reduced RBC units that were transfused. The median age of RBCs transfused to survivors was 17 days (range, 5 to 35 days) compared with 25 days (range, 9 to 36 days) for nonsurvivors ($p < 0.0001$).³⁶

Moore et al,⁶⁵ in a more recent prospective cohort study of 513 trauma patients, found that transfusion was an independent risk factor for postinjury multiple-organ failure, and described a clear dose-response relationship between the number of units transfused and the development of multiple-organ failure. Similarly Zallen and colleagues³⁷ demonstrated that in polytrauma patients the mean age of the blood, the number of units that were > 14 days old, and the number of units that were > 21 days old were all independent risk factors for multiple-organ failure.

However, two retrospective cohort studies^{66,67} subsequently were unable to show an association between the age of stored transfused RBCs and postoperative length of stay in the hospital or ICU or with the duration of postoperative mechanical ventilation. The authors attributed this lack of effect to differences in the patient population, as both studies evaluated routine postoperative coronary artery bypass graft (CABG) surgery patients, whereas Martin et al⁶³ studied a heterogeneous group of critically ill patients, which excluded cardiac surgical patients.

More recently, Vamvakas and Carven⁶⁸ reexamined the CABG population, studying the effects of RBC supernatant, platelet supernatant, and plasma components on the duration of postoperative mechanical ventilation. Their results suggested an association between RBC supernatant volume and prolonged mechanical ventilation. Proinflammatory substances that accumulate during the storage of RBC concentrates were implicated in impairing pulmonary function in these patients.^{57,68}

In a study⁶⁷ of postoperative cardiac surgery patients, the transfusion of RBCs stored for > 28 days was an independent predictor of nosocomial pneumonia. Similarly, a prospective cohort study⁶⁹ of trauma patients demonstrated that the age of transfused blood was an independent risk factor for the development of major infections. The risk of major infection increased 13% for each unit that was > 14 days old, with the most common nosocomial infection being pneumonia.⁶⁹

IMMUNOLOGY OF BLOOD TRANSFUSION

Allogenic blood transfusions introduce a multitude of foreign antigens including HLA class II-bearing donor dendritic antigen-presenting cells (APCs) in

recipients.⁵³ The normal immune response to any foreign antigen is initiated by the recognition of foreign antigens associated with the major histocompatibility complex (MHC) by host T lymphocytes (Fig 1). After encountering the antigen, naive T cells receive the first signal through the T-cell receptor-MHC plus antigenic peptide complex and received

the second signal through positive costimulatory molecules leading to full activation. Negative T-cell costimulatory pathways, on the other hand, tend to down-regulate immune responses.⁷⁰ Studies have indicated that costimulatory signals derived through non-MHC molecules that are present on APCs are required to elicit an immune response. Molecules

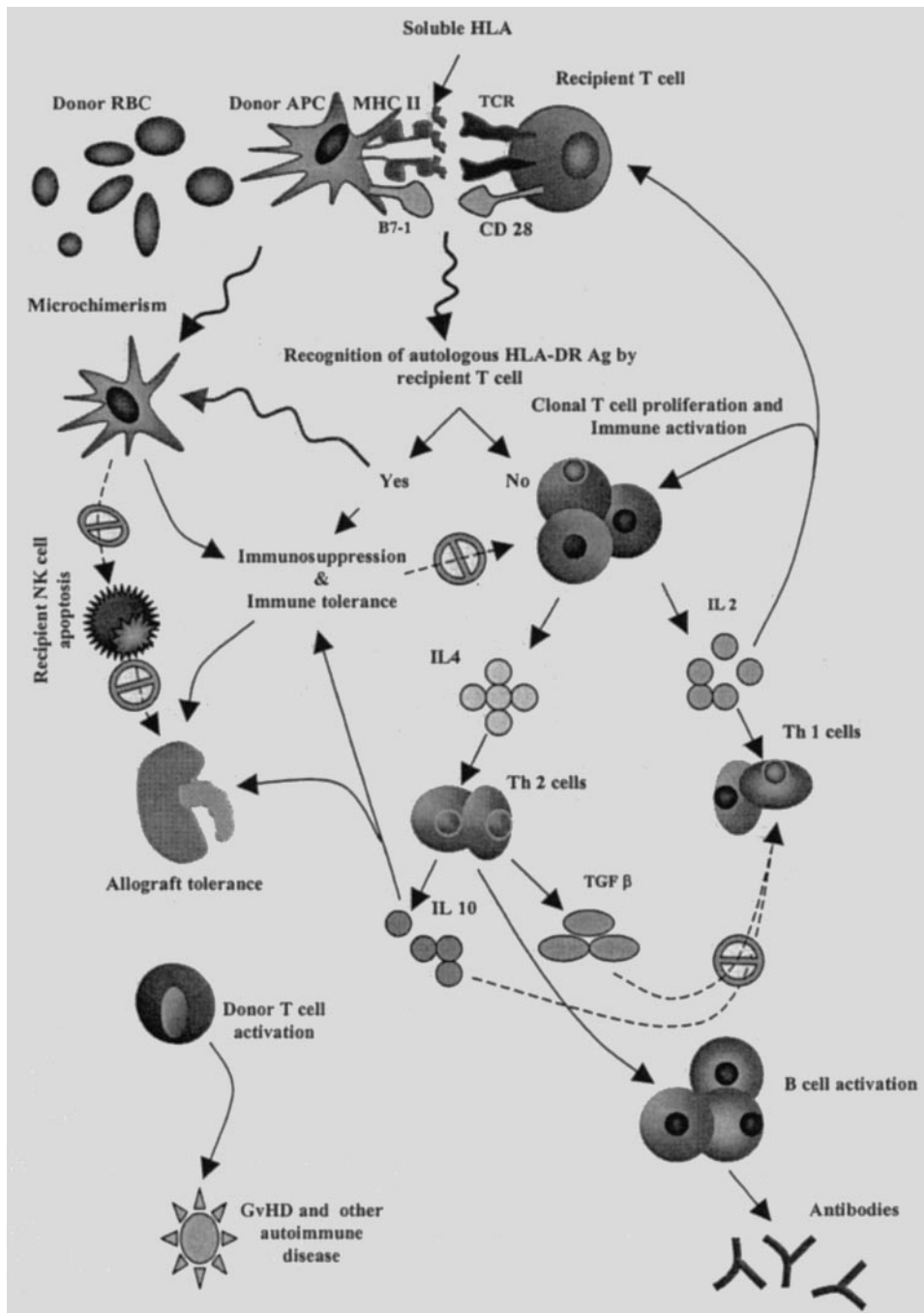


FIGURE 1. TRIM resulting from the interaction between donor APCs and recipient T cells. Recognition of donor HLA molecules as autologous antigens by recipient T cells results in immune tolerance and immunosuppression. Nonrecognition results in immune activation. Microchimerism results in immune suppression, allograft tolerance, GVHD, and autoimmune disease.

such as B7-1 and B7-2 have been shown to provide critical early costimulatory signals through CD28 and CTLA-4 T-cell receptors, which regulate IL-2 secretion and clonal T-cell proliferation.^{71,72} Various T-cell-antigen interactions induce the production of cytokines such as IL-2 and IL-4, which in turn activate T helper (Th) type 1 (IL-2) and Th-2 (IL-4) subsets, respectively. Th-2 in turn activates B-cell proliferation and antibody production. Thus, the immunogenicity of soluble, particulate, or cellular MHC antigens that are present on transfused allogenic blood products depend on the viability of APCs, the presence of costimulatory molecules to present them to recipient T cells, and HLA compatibility between donor and recipient. The impairment of any of these pathways, including the costimulatory molecules, has been shown to result in T-cell unresponsiveness.^{70,72,73}

Transfusion-Related Immunomodulation

Blood transfusions primarily induce immunomodulation in two opposite ways. They may cause either alloimmunization or tolerance induction (Fig 1). Clinical syndromes associated with immune activation in the recipient include a variety of transfusion reactions, transfusion-associated graft-vs-host disease (TAGVHD), transfusion-related lung injury (TRALI), alloimmunization, and the possible development of various autoimmune diseases. Syndromes associated with tolerance induction and immunosuppression include increased predisposition to nosocomial and postoperative infections, cancer recurrence, microchimerism, and enhanced survival of various allografts in recipients. Immunization is reflected by the induction of HLA alloantibodies and T-cell activation, while the induction of tolerance is suggested by enhanced renal, hepatic, cardiac, pancreatic, and skin allograft survival in transfused vs nontransfused recipients.⁷⁴⁻⁸² The presence or absence of autologous HLA-DR antigens on the leukocytes of the transfusion donor plays a decisive role in whether immunization or immune suppression will ensue following allogenic blood transfusion.⁸³ Transfusions sharing at least one HLA-DR antigen with the recipient will induce tolerance, while fully HLA-DR-mismatched transfusions lead to immunization.⁸⁴ The importance of the degree of HLA-DR sharing suggests a central role for CD4+ regulatory T cells. However, when a multitude of antigens is introduced into the host by blood transfusions, the host response to some of these antigens is often decreased, and immune tolerance ensues.⁵⁴

Although the exact mechanisms underlying TRIM still remains to be elucidated, allogenic blood transfusions have been shown to cause a decrease in the

helper/suppressor T-lymphocyte ratio, a decrease in natural killer cell function, defective antigen presentation, the suppression of lymphocyte blastogenesis, and a reduction in delayed-type hypersensitivity and allograft tolerance.⁸⁵⁻⁸⁷ Various bioactive soluble mediators are released from stored WBCs into human plasma during storage in a time-dependent manner as the WBCs deteriorate.⁸⁸ The concentrations of histamine, eosinophil cationic protein, eosinophilic protein X, myeloperoxidase, and plasminogen activator inhibitor-1 have all been reported to increase by threefold to 25-fold in the supernatant fluid of RBC components between day 0 and day 35 of storage.^{89,90} Histamine, eosinophil cationic protein, and eosinophil protein X have been shown to inhibit neutrophil function, thereby contributing to the development of immune suppression and tissue damage.^{91,92}

MICROCHIMERISM

Microchimerism has been proposed as a possible mechanism of TRIM in allogenic transfusions.^{55,93-96} HLA compatibility between donor and recipient blood may result in the persistence of donor leukocytes and dendritic APCs within the recipient (microchimerism). It has been postulated that such chimerism may cause the down-regulation of the recipient's immune response, including tolerance to donor alloantigens and allograft survival (Fig 1). Many years after pregnancy, liver transplantation, and neonatal exchange transfusions, microchimerism has been demonstrated, indicating a tolerance between donor and recipient cells. Microchimerism results in the release of IL-4, IL-10, and transforming growth factor- β from Th-2 lymphocytes.⁹⁷ These cytokines have been shown to inhibit the production of Th-1 cells and proinflammatory cytokines, and to deactivate cytotoxic cells, thereby suppressing allograft rejection. Dendritic APCs also have been shown to cause recipient T-cell hyporeactivity, anergy, and depletion, thus mediating immunosuppression.⁹⁸ In such immunosuppressed recipients, microchimerism may result in the development of TAGVHD, polymorphous eruption of pregnancy, and other autoimmune connective tissue diseases such as scleroderma.^{10,99,100} The loss of immunogenicity by transfused leukocytes in blood stored for > 2 weeks results in recipient T-cell anergy, thus potentiating immunodepression.¹⁰¹

DELETERIOUS EFFECTS OF RBC TRANSFUSION IN CRITICALLY ILL PATIENTS

Transfusion Transmitted Infections

Transfusion-transmitted infections due to a variety of agents, although rare, remain a cause of concern

Table 1—Potential Transfusion-Transmitted Infections and Adverse Effects of Leukocytes During Allogenic Blood Component Transfusion

Type of Infection	Infection	
Transfusion-transmitted infections	CMV	
	Epstein-Barr virus	
	West Nile virus	
	Human herpes virus-6,7,8	
	Parvovirus B19	
	Human T-cell leukemia/lymphoma virus-I and II	
	HIV 1 and 2	
	Hepatitis B and C	
	<i>Toxoplasma gondii</i>	
	<i>Trypanosoma cruzi</i>	
	Babesiosis	
	Newer agents	TTV
		SEN-V
Adverse effects of leukocyte contamination	Febrile nonhemolytic transfusion reactions	
	Refractoriness to platelet transfusions	
	TRALI	
	TAGVHD	
	Immune suppression and allograft tolerance	
	Development of possible autoimmune diseases	

in modern allogenic transfusion practice^{102,103} (Tables 1, 2). Leukocyte contamination of blood products remains the primary etiologic mode of transmission of various infectious agents. Transfusion-transmitted cytomegalovirus (CMV) occurs in approximately 4% of transfusions and is due to the reactivation of latent CMV in leukocytes from healthy donors.^{104,105} Besides CMV, other herpes viruses such as Epstein-Barr virus, human herpes virus-6, human herpes virus-7, and human herpes

virus-8 are associated with leukocyte contamination during transfusion.⁵² Human T-cell leukemia/lymphoma virus (types I and II) targets T lymphocytes and is solely transmitted by cellular blood components.¹⁰⁶ Primary toxoplasmosis has been reported¹⁰⁷ to be transmitted by whole-blood, granulocyte transfusions and from transplantation of organs from seropositive donors to immunocompromised recipients. Although theoretical concerns exist regarding the possible transmission of Creutzfeldt-Jakob disease (CJD) and new-variant CJD by blood and leukocyte transfusion, newer epidemiologic studies¹⁰⁸ have failed to show a link between the transfusion and transmission of CJD. Transfusion-transmitted West Nile virus infection occurred in the United States in 2002 among 23 patients from 14 donors, and since then > 600 infected units of blood were identified from a 2.5-million donor pool.^{102,109}

TT virus (TTV) is a novel, newly discovered DNA virus that is transmitted by transfusion to approximately 30% of patients who undergo cardiac surgery.¹¹⁰ Certain genotypes of transfusion-transmitted TTV also have been implicated in the development of hepatitis and possibly in hepatocellular carcinoma.¹¹¹ SEN virus (SEN-V) is a transfusion-transmitted DNA virus that is closely related to the TTV family. A limited number of studies¹¹² have indicated that approximately 2% of current and pre-1990 blood donors test positive for SEN-V. Although SEN-V has the potential to replicate in the liver, currently no causal relationship exists between transfusion-transmitted SEN-V infection and the development of non-A-E hepatitis.^{113,114}

Nosocomial Infections

Several studies^{8,64,65,115-120} have clearly identified the increased risk of nosocomial infections among

Table 2—Estimated Risks of Transfusion-Associated Diseases Among Immunocompetent Patients in United States*

Risk of Allogenic Transfusions	Estimated Frequency		Deaths Per Million U, No.
	Per Million U	Per Actual U	
Infection			
Hepatitis A	1	1/1,000,000	0
Hepatitis B	7-32	1/30,000-1/250,000	0-0.14
Hepatitis C	4-36	1/30,000-1/150,000	0.5-17
HIV	0.4-5	1/200,000-1/2,000,000	0.5-5
HTLV types I and II	0.5-4	1/250,000-1/2,000,000	0
Parvovirus B 19	100	1 /10,000	0
Bacterial contamination			
RBCs	2	1/500,000	0.1-0.25
Platelets	83	1/12,000	21
Acute hemolytic reactions	1-4	1/250,000-1/1,000,000	0.67
Delayed hemolytic reactions	1,000	1/1,000	0.4
TRALI	200	1/5,000	0.2

*From Goodnough et al.¹⁰³ HTLV = human T-cell lymphotropic virus.

critically ill transfused patients. Currently, there are substantial data suggesting that exposure to allogenic leukocytes in transfusions may trigger an immune system response in the recipients, leading to an increased risk of infection, an earlier recurrence of malignancy, and an increased likelihood of mortality.¹⁴ Four possible mechanisms have been attributed to the development of bacterial infections following allogenic transfusions. These include the following: (1) a TRIM effect mediated by immunologically active allogenic WBCs that down-regulate the immune function of recipients^{50,101,121}; (2) a TRIM effect mediated by soluble biological response modifiers that are released in a time-dependent manner from WBC granules or membranes into the supernatant fluid of RBCs during storage^{90,121}; (3) a TRIM effect mediated by soluble HLA peptides or other soluble mediators that circulate in allogenic plasma¹²²; and (4) a possible non-TRIM effect causing postoperative organ dysfunction that predisposes patients to infections.^{57,122-124}

Nichols and colleagues¹¹⁵ published one of the first reports that linked transfusion with an increased incidence of infection in postoperative trauma patients with intestinal perforation and documented that the number of blood transfusions positively correlated with the postoperative infection rate. Similarly, Edna and Bjerkeset¹¹⁶ found an association between RBC transfusions and infectious complications in 484 patients with acute injuries. Infectious complications developed in 46 patients (9.5%). Logistic regression analysis revealed a relationship between blood transfusions and infectious morbidity that was independent of the other significant factors, including the injury severity score, age, and surgical procedure. The corrected odds ratios for infection were 1.6 (95% confidence interval [CI], 0.7 to 3.7) when 1 to 4 U blood were given and 6.4 (95% CI, 2.3 to 18.3) when > 4 U were used.¹¹⁶

Vamvakas and Carven⁶⁴ reported that colorectal surgery patients receiving perioperative allogenic blood transfusions have strikingly longer hospital stays than similar patients who do not receive transfusions. Length of stay increased by 1.3% (95% CI, 0.5 to 2.1%) per unit of RBCs and/or platelets transfused ($p < 0.001$), and hospital charges increased by 2.0% (95% CI, 1.4 to 2.6%) per unit ($p < 0.001$). In this study, allogenic transfusions were independently associated with longer hospital stays and higher hospital charges.⁶⁴ In two separate studies,^{125,126} postoperative infection rates were similarly increased in transfused patients undergoing colorectal surgery secondary to trauma and cancer.

Braga and colleagues¹¹⁷ found that the transfusion of 1,000 mL of blood was an independent risk factor in the development of postoperative infection in

patients undergoing operations for GI cancer. Ottino et al¹¹⁸ documented that RBC transfusion was an independent risk factor for sternal wound infection in 2,579 consecutive open-heart procedures. Similarly, several studies^{35,127,128} have identified a higher risk for postoperative wound infections and pneumonia in patients undergoing CABG surgery following RBC transfusions in a dose-dependent manner. Patients with arm fractures or open leg fractures and burn patients have an increased risk of infection with transfusion when compared to nontransfused patients.^{119,120} Carson et al,¹²⁹ in a retrospective analysis, identified a 35% increased risk of nosocomial infections and a 52% increased risk of pneumonia in 9,598 hip fracture patients undergoing hip surgery who received allogenic RBC transfusions. Similarly, a linear trend between the number of units of RBCs transfused and the incidence of multiple-organ failure and infections also has been reported in trauma patients.^{37,65,130}

In critically ill patients, Taylor et al⁸ have demonstrated an association between RBC transfusion and nosocomial infection and mortality in a retrospective analysis of 1,717 patients. They investigated the rate of nosocomial infections in patients who had been admitted to a single medical-surgical-trauma unit over a period of approximately 2 years. A total of 416 patients (24%) received ≥ 1 U packed RBCs. The rate of nosocomial infection was strikingly higher and statistically significant in the group of patients who received transfusions compared with those who did not (15.4% vs 2.9%, respectively; $p < 0.005$). Moreover, a positive association was found between the number of transfusions and the incidence of nosocomial infections such that each unit of packed RBCs increased the risk of nosocomial infection by a factor of 1.5. Transfusion also was associated with both increased length of stay in the ICU and hospital and higher mortality rates. The relationship between transfusion and nosocomial infections persisted after the authors controlled for the probability of survival, age, and gender.

Shorr et al,¹³¹ in a secondary analysis of a large cohort of patients who received allogenic blood transfusions and mechanical ventilation, noted that transfusion independently increased the risk for ventilator-associated pneumonia. Of 4,892 subjects in the original cohort, 1,518 received mechanical ventilation for ≥ 48 h and did not have preexisting pneumonia. VAP was diagnosed in 20.5% of patients. The effect of transfusion on late-onset VAP was more pronounced (odds ratio, 2.16; 95% CI, 1.27 to 3.66) and demonstrated a positive dose-response relationship.¹³¹

Although the observational nature of these studies and the inability to control for all possible factors

make it difficult to establish a cause-and-effect relationship and to separate the effects of transfusion from those of the underlying condition, the results of several prospective and randomized studies^{132–134} have supported these findings. In these studies, the underlying hypothesis links the immunodepressant effect of transfusion to the presence of leukocytes (or leukocyte products).

These data have in turn led to the hypothesis that giving patients transfusions with leukocyte-reduced blood should result in reduced morbidity and mortality compared with patients receiving transfusions with non-leukocyte-reduced blood. However, most of the studies bearing on these questions have been flawed by retrospective design and inadequate consideration of the effects of comorbidities, whereas the few prospective studies in specific patient populations have reached contradictory conclusions. Metaanalyses of these substantial studies^{134–137} have failed to identify a statistically significant effect of leukocyte reduction. However, a recent study¹³⁸ evaluating clinical outcomes after the institution of a universal leukocyte reduction program in Canada noted a reduction in hospital mortality after the introduction of this program.

TRALI

TRALI is a life-threatening complication of allogenic transfusions and is the third most common cause of transfusion-associated death in the United States.¹³⁹ The estimated prevalence of TRALI is 1 in 1,120 cellular component transfusions with a mortality rate ranging from 1 to 10%.¹⁴⁰ Passively transferred donor blood containing antileukocyte antibodies (*ie*, IgG) directed against recipient leukocytes causes pulmonary sequestration, complement activation, and lung injury.¹⁴¹ In many cases, donor anti-HLA class II and antimonocyte antibodies are present.^{142,143} Stored blood containing neutrophil-activating biological response modifiers such as bioactive lipids (lysophosphatidylcholines) and cytokines such as IL-6 and IL-8 predispose the patient to microcirculatory capillary lung injury.^{123,140}

TAGVHD

TAGVHD is a rare but lethal complication with a mortality rate > 90%, in which immunocompetent donor cells proliferate and attack host hemopoietic cells, skin, liver, and bile duct epithelial cells. Although more common in immunocompromised patients, the risk factors for the development of graft-vs-host disease (GVHD) include patients receiving transfusions from HLA-homozygous donors who are haploidentical with the patient, the use of relatives as donors, male recipients, and fresh blood containing

viable lymphocytes.⁵⁴ Homozygosity for HLA occurs in 2% of the population, and the estimated chance of transfusion of an HLA homozygous product that is haploidentical with the patient is 1 in 800 patients.¹⁴⁴ Thus, only a small fraction of such transfusions cause GVHD. However, it is now apparent that even histoincompatible WBCs circulate for up to a week in heavily transfused patients and thus have a potential to cause subclinical GVHD.¹⁴⁵ Irradiated and leukodepleted cellular products avert the development of GVHD.¹⁴⁶

Leukodepletion and Transfusion

Transfused leukocytes have been implicated in a variety of biological effects including febrile nonhemolytic transfusion reactions, transfusion-related alloimmunization to platelets, TRALI, and GVHD (Table 1). The potential beneficial effects of universal leukocyte depletion include a reduction in the incidence of nonhemolytic transfusion reactions, immunosuppression, and mortality.^{138,147–150} Both autologous and allogenic nonleukodepleted blood components release soluble bioactive mediators during storage, thereby mediating some of the TRIM effects, while the prestorage leukodepletion of allogenic cellular products has been shown to prevent some of its deleterious effects.^{50,51,88,90,134,150} As prestorage leukodepletion is essential to prevent the accumulation of biological response modifiers, some studies comparing the incidence of postoperative infections,¹⁵¹ and cancer recurrence¹⁵² between the allogenic and autologous transfusions have reported similar outcomes, independent of the RBC component that was transfused.³⁵ It is also noteworthy that the negative effects of a liberal transfusion strategy observed in the Transfusion Requirements in Critical Care study³⁹ predated the implementation of universal leukoreduction in Canada. It is therefore conceivable that the detrimental effects of blood transfusion may be mediated primarily by the donor WBCs and their complex interactions with stored RBCs, rather than by the RBCs alone.

CONCLUSION

Concerns regarding the excess morbidity and mortality associated with nonrestrictive transfusion strategies, coupled with the emerging increased risk of the transmission of newer infectious agents, and immunomodulation, should prompt the reevaluation of current transfusion protocols in critically ill patients. A restrictive transfusion strategy appears to improve outcomes in critically ill patients. Lowering the trigger for transfusion to a hemoglobin concentration of approximately 7 g/dL in patients without

coronary disease and implementing other blood conservation techniques, such as minimizing phlebotomy, the reuse of discarded blood by using closed circuits of blood sampling, and the use of recombinant EPO, should help to lower transfusion requirements. In addition, the implementation of prestorage leukodepleted blood, along with pathogen inactivation techniques may reduce the adverse effects associated with allogenic transfusions. The benefit of fresh leukodepleted blood (*ie*, < 15 days) compared to leukodepleted old blood (*ie*, > 15 days) has yet to be determined. However, the major clinical dilemma is not between the use of fresh vs old blood and/or leukocyte-depleted vs non-leukocyte-depleted blood but between stored blood vs no blood.

REFERENCES

- 1 Wells AW, Mounter PJ, Chapman CE, et al. Where does blood go? Prospective observational study of red cell transfusion in north England. *BMJ* 2002; 325:803
- 2 National Blood Data Resource Center. Comprehensive report on blood collection and transfusion in the United States. Bethesda, MD: National Blood Data Resource Center, 2001
- 3 Groeger JS, Guntupalli KK, Strosberg M, et al. Descriptive analysis of critical care units in the United States: patient characteristics and intensive care unit utilization. *Crit Care Med* 1993; 21:279–291
- 4 Corwin HL. Blood transfusion in the critically ill patient. *Dis Mon* 1999; 45:409–426
- 5 Cane RD. Hemoglobin: how much is enough? *Crit Care Med* 1990; 18:1046–1047
- 6 Hebert PC, Fergusson DA. Red blood cell transfusions in critically ill patients. *JAMA* 2002; 288:1525–1526
- 7 Marik PE, Sibbald WJ. Effect of stored-blood transfusion on oxygen delivery in patients with sepsis. *JAMA* 1993; 269:3024–3029
- 8 Taylor RW, Manganaro L, O'Brien J, et al. Impact of allogenic packed red blood cell transfusion on nosocomial infection rates in the critically ill patient. *Crit Care Med* 2002; 30:2249–2254
- 9 Vincent JL, Baron JF, Reinhart K, et al. Anemia and blood transfusion in critically ill patients. *JAMA* 2002; 288:1499–1507
- 10 Martin L, Watier H, Vaillant L, et al. Sjogren's syndrome and vitiligo in a woman with posttransfusion microchimerism. *Am J Med* 2001; 111:419–421
- 11 Corwin HL, Rodriguez RM, Pearl RG, et al. Erythropoietin response in critically ill patients [abstract]. *Crit Care Med* 1997; 25:A82
- 12 von Ahsen N, Muller C, Serke S, et al. Important role of nondiagnostic blood loss and blunted erythropoietic response in the anemia of medical intensive care patients. *Crit Care Med* 1999; 27:2630–2639
- 13 Brown RB, Klar J, Teres D, et al. Prospective study of clinical bleeding in intensive care unit patients. *Crit Care Med* 1988; 16:1171–1176
- 14 Corwin HL, Parsonnet KC, Gettinger A. RBC transfusion in the ICU: is there a reason? *Chest* 1995; 108:767–771
- 15 Smoller BR, Kruskall MS. Phlebotomy for diagnostic laboratory tests in adults: pattern of use and effect on transfusion requirements. *N Engl J Med* 1986; 314:1233–1235
- 16 van Iperen CE, Gaillard CA, Kraaijenhagen RJ, et al. Response of erythropoiesis and iron metabolism to recombinant human erythropoietin in intensive care unit patients. *Crit Care Med* 2000; 28:2773–2778
- 17 Batge B, Filejski W, Kurowski V, et al. Clostridial sepsis with massive intravascular hemolysis: rapid diagnosis and successful treatment. *Intensive Care Med* 1992; 18:488–490
- 18 Campillo B, Zittoun J, de Gialluly E. Prophylaxis of folate deficiency in acutely ill patients: results of a randomized clinical trial. *Intensive Care Med* 1988; 14:640–645
- 19 Rodriguez RM, Corwin HL, Gettinger A, et al. Nutritional deficiencies and blunted erythropoietin response as causes of the anemia of critical illness. *J Crit Care* 2001; 16:36–41
- 20 Corwin HL, Gettinger A, Pearl RG, et al. Efficacy of recombinant human erythropoietin in critically ill patients: a randomized controlled trial. *JAMA* 2002; 288:2827–2835
- 21 Elliot JM, Virankabutra T, Jones S, et al. Erythropoietin mimics the acute phase response in critical illness. *Crit Care* 2003; 7:R35–R40
- 22 Krafte-Jacobs B, Levetown ML, Bray GL, et al. Erythropoietin response to critical illness. *Crit Care Med* 1994; 22:821–826
- 23 Krafte-Jacobs B. Anemia of critical illness and erythropoietin deficiency. *Intensive Care Med* 1997; 23:137–138
- 24 Rogiers P, Zhang H, Leeman M, et al. Erythropoietin response is blunted in critically ill patients. *Intensive Care Med* 1997; 23:159–162
- 25 Frede S, Fandrey J, Pagel H, et al. Erythropoietin gene expression is suppressed after lipopolysaccharide or interleukin-1 beta injections in rats. *Am J Physiol* 1997; 273:R1067–R1071
- 26 Jekmann WE, Fandrey J, Frede S, et al. Inhibition of erythropoietin production by cytokines: implications for the anemia involved in inflammatory states. *Ann N Y Acad Sci* 1994; 718:300–309
- 27 Johnson CS, Keckler DJ, Topper MI, et al. *In vivo* hematopoietic effects of recombinant interleukin-1 α in mice: stimulation of granulocytic, monocytic, megakaryocytic, and early erythroid progenitors, suppression of late-stage erythropoiesis, and reversal of erythroid suppression with erythropoietin. *Blood* 1989; 73:678–683
- 28 Johnson CS, Cook CA, Furmanski P. *In vivo* suppression of erythropoiesis by tumor necrosis factor-alpha (TNF- α): reversal with exogenous erythropoietin (EPO). *Exp Hematol* 1990; 18:109–113
- 29 Fishbane S. Review of issues relating to iron and infection. *Am J Kidney Dis* 1999; 34:S47–S52
- 30 Jurado RL. Iron, infections, and anemia of inflammation. *Clin Infect Dis* 1997; 25:888–895
- 31 Balla G, Vercellotti GM, Muller-Eberhard U, et al. Exposure of endothelial cells to free heme potentiates damage mediated by granulocytes and toxic oxygen species. *Lab Invest* 1991; 64:648–655
- 32 Fernandes CJ Jr, Akamine N, De Marco FV, et al. Red blood cell transfusion does not increase oxygen consumption in critically ill septic patients. *Crit Care* 2001; 5:362–367
- 33 Gramm J, Smith S, Gamelli RL, et al. Effect of transfusion on oxygen transport in critically ill patients. *Shock* 1996; 5:190–193
- 34 Mynster T, Nielsen HJ. The impact of storage time of transfused blood on postoperative infectious complications in rectal cancer surgery: Danish RANX05 Colorectal Cancer Study Group. *Scand J Gastroenterol* 2000; 35:212–217
- 35 Vamvakas EC, Carven JH. Transfusion and postoperative pneumonia in coronary artery bypass graft surgery: effect of the length of storage of transfused red cells. *Transfusion* 1999; 39:701–710

- 36 Purdy FR, Tweeddale MG, Merrick PM. Association of mortality with age of blood transfused in septic ICU patients. *Can J Anaesth* 1997; 44:1256–1261
- 37 Zallen G, Offner PJ, Moore EE, et al. Age of transfused blood is an independent risk factor for postinjury multiple organ failure. *Am J Surg* 1999; 178:570–572
- 38 Corwin HL, Gettinger A, Pearl RG, et al. The CRIT Study: anemia and blood transfusion in the critically ill; current clinical practice in the United States. *Crit Care Med* 2004; 32:39–52
- 39 Hebert PC, Wells G, Blajchman MA, et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care: Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. *N Engl J Med* 1999; 340:409–417
- 40 Hebert PC, Yetisir E, Martin C, et al. Is a low transfusion threshold safe in critically ill patients with cardiovascular diseases? *Crit Care Med* 2001; 29:227–234
- 41 Hebert PC, Wells G, Marshall J, et al. Transfusion requirements in critical care: a pilot study; Canadian Critical Care Trials Group. *JAMA* 1995; 273:1439–1444
- 42 Apstein CS, Dennis RC, Briggs L, et al. Effect of erythrocyte storage and oxyhemoglobin affinity changes on cardiac function. *Am J Physiol* 1985; 248:H508–H515
- 43 Fischer DJ, Torrence NJ, Sprung RJ, et al. Determination of erythrocyte deformability and its correlation to cellular ATP release using microbore tubing with diameters that approximate resistance vessels *in vivo*. *Analyst* 2003; 128:1163–1168
- 44 Stuart J, Nash GB. Red cell deformability and haematological disorders. *Blood Rev* 1990; 4:141–147
- 45 Simchon S, Jan KM, Chien S. Influence of reduced red cell deformability on regional blood flow. *Am J Physiol* 1987; 253:H898–H903
- 46 Luk CS, Gray-Statchuk LA, Cepinkas G, et al. WBC reduction reduces storage-associated RBC adhesion to human vascular endothelial cells under conditions of continuous flow *in vitro*. *Transfusion* 2003; 43:151–156
- 47 Ho J, Milkovic S, Gray L, et al. Transfusion of stored red blood cells (RBC) occlude the rat microvasculature *in vivo*. *Blood* 2001; 98:544a
- 48 Wolfe LC, Byrne AM, Lux SE. Molecular defect in the membrane skeleton of blood bank-stored red cells: abnormal spectrin-protein 4.1-actin complex formation. *J Clin Invest* 1986; 78:1681–1686
- 49 Racek J, Herynkova R, Holecek V, et al. Influence of antioxidants on the quality of stored blood. *Vox Sang* 1997; 72:16–19
- 50 Bordin JO, Heddle NM, Blajchman MA. Biologic effects of leukocytes present in transfused cellular blood products. *Blood* 1994; 84:1703–1721
- 51 Jensen LS, Kissmeyer-Nielsen P, Wolff B, et al. Randomised comparison of leucocyte-depleted versus buffy-coat-poor blood transfusion and complications after colorectal surgery. *Lancet* 1996; 348:841–845
- 52 Chu RW. Leukocytes in blood transfusion: adverse effects and their prevention. *Hong Kong Med J* 1999; 5:280–284
- 53 Austyn JM. Antigen uptake and presentation by dendritic leukocytes. *Semin Immunol* 1992; 4:227–236
- 54 Brand A. Immunological aspects of blood transfusions. *Transpl Immunol* 2002; 10:183–190
- 55 Dzik WH. Mononuclear cell microchimerism and the immunomodulatory effect of transfusion. *Transfusion* 1994; 34:1007–1012
- 56 Stack G, Baril L, Napychank P, et al. Cytokine generation in stored, white cell-reduced, and bacterially contaminated units of red cells. *Transfusion* 1995; 35:199–203
- 57 Fransen E, Maessen J, Dentener M, et al. Impact of blood transfusions on inflammatory mediator release in patients undergoing cardiac surgery. *Chest* 1999; 116:1233–1239
- 58 Mynster T, Dybkjoer E, Kronborg G, et al. Immunomodulating effect of blood transfusion: is storage time important? *Vox Sang* 1998; 74:176–181
- 59 Zallen G, Moore EE, Ciesla DJ, et al. Stored red blood cells selectively activate human neutrophils to release IL-8 and secretory PLA2. *Shock* 2000; 13:29–33
- 60 Hogman CF, Meryman HT. Storage parameters affecting red blood cell survival and function after transfusion. *Transfus Med Rev* 1999; 13:275–296
- 61 d'Almeida MS, Jagger J, Duggan M, et al. A comparison of biochemical and functional alterations of rat and human erythrocytes stored in CPDA-1 for 29 days: implications for animal models of transfusion. *Transfus Med* 2000; 10:291–303
- 62 Prins HA, Houdijk AP, Nijveldt RJ, et al. Arginase release from red blood cells: possible link in transfusion induced immune suppression? *Shock* 2001; 16:113–115
- 63 Martin CM, Sibbald WJ, Lu X, et al. Age of transfused red blood cells is associated with ICU length of stay. *Clin Invest Med* 1994; 17:B21a
- 64 Vamvakas EC, Carven JH. Allogeneic blood transfusion, hospital charges, and length of hospitalization: a study of 487 consecutive patients undergoing colorectal cancer resection. *Arch Pathol Lab Med* 1998; 122:145–151
- 65 Moore FA, Moore EE, Sauaia A. Blood transfusion: an independent risk factor for postinjury multiple organ failure. *Arch Surg* 1997; 132:620–624
- 66 Vamvakas EC, Carven JH. Length of storage of transfused red cells and postoperative morbidity in patients undergoing coronary artery bypass graft surgery. *Transfusion* 2000; 40:101–109
- 67 Leal-Noval SR, Jara-Lopez I, Garcia-Garmendia JL, et al. Influence of erythrocyte concentrate storage time on post-surgical morbidity in cardiac surgery patients. *Anesthesiology* 2003; 98:815–822
- 68 Vamvakas EC, Carven JH. Allogeneic blood transfusion and postoperative duration of mechanical ventilation: effects of red cell supernatant, platelet supernatant, plasma components and total transfused fluid. *Vox Sang* 2002; 82:141–149
- 69 Offner PJ, Moore EE, Biffi WL, et al. Increased rate of infection associated with transfusion of old blood after severe injury. *Arch Surg* 2002; 137:711–716
- 70 Sho M, Sandner SE, Najafian N, et al. New insights into the interactions between T-cell costimulatory blockade and conventional immunosuppressive drugs. *Ann Surg* 2002; 236:667–675
- 71 Freeman GJ, Gribben JG, Boussiotis VA, et al. Cloning of B7-2: a CTLA-4 counter-receptor that costimulates human T cell proliferation. *Science* 1993; 262:909–911
- 72 Rothstein DM, Sayegh MH. T-cell costimulatory pathways in allograft rejection and tolerance. *Immunol Rev* 2003; 196:85–108
- 73 Mincheff MS, Meryman HT. Costimulatory signals necessary for induction of T cell proliferation. *Transplantation* 1990; 49:768–772
- 74 Anderson CB, Brennan D, Keller C, et al. Beneficial effects of donor-specific transfusions on long-term renal allograft function. *Transplant Proc* 1995; 27:991–994
- 75 Flye MW, Burton K, Mohanakumar T, et al. Donor-specific transfusions have long-term beneficial effects for human renal allografts. *Transplantation* 1995; 60:1395–1401
- 76 Inuzuka S, Koga S, Nishikido M, et al. Donor-specific blood transfusion prolongs cardiac allograft survival in rats by low

- nitric oxide production and elevated serum levels of prostaglandin E(2). *Immunol Lett* 2002; 83:119–124
- 77 Jasklowska-Englisz M, Olszewski WL. Donor-specific transfusions prolong heart allograft survival but lymphocytes from the same donor are acutely rejected. *Transplant Proc* 1994; 26:3463
 - 78 Liang J, Yamaguchi Y, Matsuda T, et al. Posttransplant infusion of donor-specific blood induces immunological unresponsiveness in rat hepatic allografts. *Transplantation* 2000; 70:1363–1371
 - 79 Muller CJ, du Toit DF, Page BJ, et al. Peritransplant donor-specific transfusion combined with anti-CD4 and cyclosporine induction therapy prolongs foetal rat pancreas allograft survival. *Transplant Proc* 2002; 34:2889–2890
 - 80 Rifle G, Mousson C. Donor-derived hematopoietic cells in organ transplantation: a major step toward allograft tolerance? *Transplantation* 2003; 75:3S–7S
 - 81 Roelen D, Brand A, Claas FH. Pretransplant blood transfusions revisited: a role for CD(4+) regulatory T cells? *Transplantation* 2004; 77:S26–S28
 - 82 Xu H, Montgomery SP, Preston EH, et al. Studies investigating pretransplant donor-specific blood transfusion, rapamycin, and the CD154-specific antibody IDEC-131 in a nonhuman primate model of skin allotransplantation. *J Immunol* 2003; 170:2776–2782
 - 83 Lagaaj EL, Ruigrok MB, van Rood JJ, et al. Blood transfusion induced changes in cell-mediated lympholysis: to immunize or not to immunize. *J Immunol* 1991; 147:3348–3352
 - 84 Roelen DL, van Rood JJ, Brand A, et al. Immunomodulation by blood transfusions. *Vox Sang* 2000; 78:273–275
 - 85 Brunson ME, Alexander JW. Mechanisms of transfusion-induced immunosuppression. *Transfusion* 1990; 30:651–658
 - 86 Jensen LS, Andersen AJ, Christiansen PM, et al. Postoperative infection and natural killer cell function following blood transfusion in patients undergoing elective colorectal surgery. *Br J Surg* 1992; 79:513–516
 - 87 Blajchman MA, Bordin JO. Mechanisms of transfusion-associated immunosuppression. *Curr Opin Hematol* 1994; 1:457–461
 - 88 Humbert JR, Fermin CD, Winsor EL. Early damage to granulocytes during storage. *Semin Hematol* 1991; 28:10–13
 - 89 Frewin DB, Jonsson JR, Head RJ, et al. Histamine levels in stored human blood. *Transfusion* 1984; 24:502–504
 - 90 Nielsen HJ, Reimert CM, Pedersen AN, et al. Time-dependent, spontaneous release of white cell- and platelet-derived bioactive substances from stored human blood. *Transfusion* 1996; 36:960–965
 - 91 Bury TB, Corhay JL, Radermecker MF. Histamine-induced inhibition of neutrophil chemotaxis and T-lymphocyte proliferation in man. *Allergy* 1992; 47:624–629
 - 92 Peterson CG, Skoog V, Venge P. Human eosinophil cationic proteins (ECP and EPX) and their suppressive effects on lymphocyte proliferation. *Immunobiology* 1986; 171:1–13
 - 93 Beko KR, Tran HO, Hewitt CW, et al. Mechanisms of prior blood transfusion-cyclosporine-induced tolerance: a potential role for immune-cellular chimerism. *Transplant Proc* 1991; 23:147–148
 - 94 Dzik WH. Microchimerism after transfusion: the spectrum from GVHD to alloimmunization. *Transfus Sci* 1995; 16: 107–108
 - 95 Fisher RA, Cohen DS, Ben Ezra JM, et al. Induction of long-term graft tolerance and donor/recipient chimerism. *J Surg Res* 1996; 60:181–185
 - 96 Tajik N, Singal D, Pourmand G, et al. Prospective study of microchimerism in renal allograft recipients: association between HLA-DR matching, microchimerism and acute rejection. *Clin Transplant* 2001; 15:192–198
 - 97 Gafter U, Kalechman Y, Sredni B. Blood transfusion enhances production of T-helper-2 cytokines and transforming growth factor beta in humans. *Clin Sci (Lond)* 1996; 91: 519–523
 - 98 Thomson AW, Lu L, Murase N, et al. Microchimerism, dendritic cell progenitors and transplantation tolerance. *Stem Cells* 1995; 13:622–639
 - 99 Aractingi S, Berkane N, Bertheau P, et al. Fetal DNA in skin of polymorphic eruptions of pregnancy. *Lancet* 1998; 352: 1898–1901
 - 100 Nelson JL. Microchimerism and human autoimmune diseases. *Lupus* 2002; 11:651–654
 - 101 Mincheff MS, Meryman HT, Kapoor V, et al. Blood transfusion and immunomodulation: a possible mechanism. *Vox Sang* 1993; 65:18–24
 - 102 Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. *N Engl J Med* 2003; 349:1236–1245
 - 103 Goodnough LT, Brecher ME, Kanter MH, et al. Transfusion medicine: first of two parts; blood transfusion. *N Engl J Med* 1999; 340:438–447
 - 104 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
 - 105 Bowden RA, Sayers M, Flournoy N, et al. Cytomegalovirus immune globulin and seronegative blood products to prevent primary cytomegalovirus infection after marrow transplantation. *N Engl J Med* 1986; 314:1006–1010
 - 106 Sandler SG, Fang CT, Williams AE. Human T-cell lymphotropic virus type I and II in transfusion medicine. *Transfus Med Rev* 1991; 5:93–107
 - 107 Siegel SE, Lunde MN, Gelderman AH, et al. Transmission of toxoplasmosis by leukocyte transfusion. *Blood* 1971; 37:388–394
 - 108 Dodd RY, Sullivan MT. Creutzfeldt-Jakob disease and transfusion safety: tilting at icebergs? *Transfusion* 1998; 38:221–223
 - 109 Centers for Disease Control and Prevention. CDC update: detection of West Nile virus in blood donation in United States. Available at: www.cdc.gov/mmwr/preview/mmwrhtml/mm52d918a1.htm. Accessed December 16, 2003
 - 110 Wang JT, Lee CZ, Kao JH, et al. Incidence and clinical presentation of posttransfusion TT virus infection in prospectively followed transfusion recipients: emphasis on its relevance to hepatitis. *Transfusion* 2000; 40:596–601
 - 111 Takacs M, Lengyel A, Dencs A, et al. Newly discovered hepatitis viruses: do they cause hepatitis indeed? *Orv Hetil* 2003; 144:1569–1574
 - 112 Chamberland ME, Alter HJ, Busch MP, et al. Emerging infectious disease issues in blood safety. *Emerg Infect Dis* 2001; 7:552–553
 - 113 Umemura T, Yeo AE, Sottini A, et al. SEN virus infection and its relationship to transfusion-associated hepatitis. *Hepatology* 2001; 33:1303–1311
 - 114 Yoshida H, Kato N, Shiratori Y, et al. Weak association between SEN virus viremia and liver disease. *J Clin Microbiol* 2002; 40:3140–3145
 - 115 Nichols RL, Smith JW, Klein DB, et al. Risk of infection after penetrating abdominal trauma. *N Engl J Med* 1984; 311:1065–1070
 - 116 Edna TH, Bjerkeset T. Association between blood transfusion and infection in injured patients. *J Trauma* 1992; 33:659–661
 - 117 Braga M, Vignali A, Radaelli G, et al. Association between

- perioperative blood transfusion and postoperative infection in patients having elective operations for gastrointestinal cancer. *Eur J Surg* 1992; 158:531–536
- 118 Ottino G, De Paulis R, Pansini S, et al. Major sternal wound infection after open-heart surgery: a multivariate analysis of risk factors in 2,579 consecutive operative procedures. *Ann Thorac Surg* 1987; 44:173–179
 - 119 Graves TA, Cioffi WG, Mason AD Jr, et al. Relationship of transfusion and infection in a burn population. *J Trauma* 1989; 29:948–952
 - 120 Dellinger EP, Miller SD, Wertz MJ, et al. Risk of infection after open fracture of the arm or leg. *Arch Surg* 1988; 123:1320–1327
 - 121 Kao KJ. Induction of humoral immune tolerance to major histocompatibility complex antigens by transfusions of UVB-irradiated leukocytes. *Blood* 1996; 88:4375–4382
 - 122 Krensky AM, Clayberger C. Structure of HLA molecules and immunosuppressive effects of HLA derived peptides. *Int Rev Immunol* 1996; 13:173–185
 - 123 Silliman CC, Voelkel NF, Allard JD, et al. Plasma and lipids from stored packed red blood cells cause acute lung injury in an animal model. *J Clin Invest* 1998; 101:1458–1467
 - 124 Nielsen HJ. Detrimental effects of perioperative blood transfusion. *Br J Surg* 1995; 82:582–587
 - 125 Sauaia A, Moore FA, Moore EE, et al. Early predictors of postinjury multiple organ failure. *Arch Surg* 1994; 129:39–45
 - 126 Dawes LG, Arahamian C, Condon RE, et al. The risk of infection after colon injury. *Surgery* 1986; 100:796–803
 - 127 Chelemer SB, Prato BS, Cox PM Jr, et al. Association of bacterial infection and red blood cell transfusion after coronary artery bypass surgery. *Ann Thorac Surg* 2002; 73:138–142
 - 128 Murphy PJ, Connery C, Hicks GL Jr, et al. Homologous blood transfusion as a risk factor for postoperative infection after coronary artery bypass graft operations. *J Thorac Cardiovasc Surg* 1992; 104:1092–1099
 - 129 Carson JL, Altman DG, Duff A, et al. Risk of bacterial infection associated with allogeneic blood transfusion among patients undergoing hip fracture repair. *Transfusion* 1999; 39:694–700
 - 130 Claridge JA, Sawyer RG, Schulman AM, et al. Blood transfusions correlate with infections in trauma patients in a dose-dependent manner. *Am Surg* 2002; 68:566–572
 - 131 Shorr AF, Duh MS, Kelly KM, et al. Red blood cell transfusion and ventilator-associated pneumonia: a potential link? *Crit Care Med* 2004; 32:666–674
 - 132 Gazmuri RJ, Shakeri SA. Blood transfusion and the risk of nosocomial infection: an underreported complication? *Crit Care Med* 2002; 30:2389–2391
 - 133 Houbiers JG, van de Velde CJ, van de Watering LM, et al. Transfusion of red cells is associated with increased incidence of bacterial infection after colorectal surgery: a prospective study. *Transfusion* 1997; 37:126–134
 - 134 van de Watering LM, Hermans J, Houbiers JG, 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
 - 135 Vamvakas EC, Blajchman MA. Deleterious clinical effects of transfusion-associated immunomodulation: fact or fiction? *Blood* 2001; 97:1180–1195
 - 136 Vamvakas EC, Blajchman MA. Universal WBC reduction: the case for and against. *Transfusion* 2001; 41:691–712
 - 137 McAlister FA, Clark HD, Wells PS, et al. Perioperative allogeneic blood transfusion does not cause adverse sequelae in patients with cancer: a meta-analysis of unconfounded studies. *Br J Surg* 1998; 85:171–178
 - 138 Hebert PC, Fergusson D, Blajchman MA, et al. Clinical outcomes following institution of the Canadian universal leukoreduction program for red blood cell transfusions. *JAMA* 2003; 289:1941–1949
 - 139 Lee JH. Transfusion-related fatalities: reports to US FDA; 1990–1998. *ABC Newsletter* 1999; 1:6
 - 140 Silliman CC, Boshkov LK, Mehdizadehkashi Z, et al. Transfusion-related acute lung injury: epidemiology and a prospective analysis of etiologic factors. *Blood* 2003; 101:454–462
 - 141 Bux J, Becker F, Seeger W, et al. Transfusion-related acute lung injury due to HLA-A2-specific antibodies in recipient and NB1-specific antibodies in donor blood. *Br J Haematol* 1996; 93:707–713
 - 142 Kopko PM, Paglieroni TG, Popovsky MA, et al. TRALI: correlation of antigen-antibody and monocyte activation in donor-recipient pairs. *Transfusion* 2003; 43:177–184
 - 143 Kopko PM, Popovsky MA, MacKenzie MR, et al. HLA class II antibodies in transfusion-related acute lung injury. *Transfusion* 2001; 41:1244–1248
 - 144 McMilin KD, Johnson RL. HLA homozygosity and the risk of related-donor transfusion-associated graft-versus-host disease. *Transfus Med Rev* 1993; 7:37–41
 - 145 Adams PT, Davenport RD, Reardon DA, et al. Detection of circulating donor white blood cells in patients receiving multiple transfusions. *Blood* 1992; 80:551–555
 - 146 Linden JV, Pisciotto PT. Transfusion-associated graft-versus-host disease and blood irradiation. *Transfus Med Rev* 1992; 6:116–123
 - 147 van de Watering LM, Brand A, Houbiers JG, et al. Perioperative blood transfusions, with or without allogeneic leukocytes, relate to survival, not to cancer recurrence. *Br J Surg* 2001; 88:267–272
 - 148 Bilgin YM, van de Watering LMG, Lorinser JE, et al. The effects of prestorage leukocyte-depletion of erythrocyte concentrates in cardiac surgery: a double-blind randomized clinical trial [abstract]. *Blood* 2001; 98:828a
 - 149 Biedler AE, Schneider SO, Seyfert U, et al. Impact of alloantigens and storage-associated factors on stimulated cytokine response in an in vitro model of blood transfusion. *Anesthesiology* 2002; 97:1102–1109
 - 150 Pruss A, Kalus U, Radtke H, et al. Universal leukodepletion of blood components results in a significant reduction of febrile non-hemolytic but not allergic transfusion reactions. *Transfus Apheresis Sci* 2004; 30:41–46
 - 151 Busch OR, Hop WC, Marquet RL, et al. Autologous blood and infections after colorectal surgery. *Lancet* 1994; 343:668–669
 - 152 Ness PM, Walsh PC, Zahurak M, et al. Prostate cancer recurrence in radical surgery patients receiving autologous or homologous blood. *Transfusion* 1992; 32:31–36