Biologic Effects of Leukocytes Present in Transfused Cellular Blood Products
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Despite careful donor selection and extensive testing, the transfusion of allogeneic cellular blood products is associated occasionally with adverse reactions and other complications. The latter include febrile nonhemolytic transfusion reactions (FNHTR), refractoriness to platelet transfusions, graft-versus-host disease (GVHD), immunomodulation, and transmission of infectious agents. There is increasing awareness that some of these may be attributable to the presence of leukocytes in donor blood. The use of leukodepleted blood components has thus been recommended as a preventative intervention for selected groups of patients who are at high risk of reaction. This review will focus on the data currently available relating to the pathogenesis of the various biologic effects associated with the presence of allogeneic leukocytes in transfused cellular blood products. In addition, available methods to prevent such complications will be discussed.

Normal Physiologic Properties of Leukocytes
Formation, Kinetics, and Function

For the purpose of this discussion, peripheral blood leukocytes will be divided into two functional groups: the phagocytes and the immunocytes. Granulocytes (neutrophils, eosinophils, and basophils) and the monocytes will be considered among the phagocytes, whereas the various types of lymphocytes, along with their precursors, and the plasma cells will be discussed as immunocytes. Both classes of leukocytes originate from a common pluripotent hematopoietic stem cell and develop into specific cell types under the influence of various growth factors generally known as cytokines. These hormone-like glycoproteins, produced by a wide variety of cells, are involved in intercellular communication. The cytokines act hierarchically and synergistically at low concentration to induce leukocyte proliferation, differentiation, maturation, and specific function. The cytokines thus influence the development and maintenance of immunity, and the inflammatory processes, by regulating the biologic interaction among the different cell types.

The cytokines are biologically active at various specific steps during the differentiation and proliferation of hematopoietic stem cells, acting as either lineage-specific factors, progenitor cell-cycling factors, or inhibitory factors. Thus, erythropoietin (EPO) regulates erythropoiesis; macrophage colony-stimulating factor (M-CSF) and interleukin-5 (IL-5) control the growth of the eosinophil and monocyte/macrophage lineages; granulocyte-CSF (G-CSF) acts on neutrophil progenitors; whereas IL-6, IL-11, IL-3, IL-1, stem cell factor (SCF), leukemia-inhibitory factor (LIF), and granulocyte-macrophage-CSF (GM-CSF) have been shown to stimulate the formation of neutrophils, macrophages, and megakaryocytes. IL-3, IL-4, and GM-CSF act on intermediate progenitors after they leave the resting state, termed G0. Several factors, including IL-3 and GM-CSF, have been shown to act synergistically to cycle dormant human hematopoietic progenitors to promote colony formation; whereas IL-6, G-CSF, IL-11, stem factor (SF), and IL-12 are active in dormant murine hematopoietic progenitors. The inhibitory cytokines include tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), macrophage inflammatory protein 1α (MIP1α), and, possibly, IL-4.

Recently, it has been recognized that there are stem cells that are progenitors for all hematopoietic lineages, including immunocytes. These stem cells are not only in the bone marrow but also in the lymphoid tissues, but those in the latter tissue appear to produce both B and T lymphocytes. B lymphocytes, originating from the bone marrow, represent approximately 20% of circulating lymphocytes. Circulating T lymphocytes also arise from the bone marrow, but these are processed in the thymus before they are distributed to the lymph nodes and spleen. Thus, approximately 80% of circulating lymphocytes are T cells that remain in the circulation for approximately 10 hours.

Dendritic leukocytes, which also originate in the hematopoietic system, participate in the immune response by acting as antigen-presenting cells (APC). T lymphocytes are able to recognize foreign antigens associated with the major histocompatibility complex (MHC) molecules (in humans, the HLA antigens), presented by the APC, to initiate the immune response. Thus, APC both present peptide-MHC complexes to T lymphocytes and deliver activation signals initiating the T-dependent immune response. This T-cell–antigen interaction induces the production of various cytokines by both APC and T cells to cause the rapid expansion and differentiation of alloantigen-specific T lymphocytes. The production of specific cytotoxic T lymphocytes is thus dependent on specific cytokines, especially IL-2 and IL-4. The IL-2 drives the differentiation of naïve T cells toward the T helper 1 (Th1) subset, which, in turn, controls cell-mediated immunity. IL-4 directs naïve T lymphocytes to become T helper 2 (Th2) cells, which, in turn, mediate antibody production by B lymphocytes. The B cell is then primed to respond to a variety of cytokines released by T helper cells that include IL-2, IL-4, IL-6, and interferon-γ (INF-γ) IL-2 together with IL-5 induces B-cell proliferation, whereas IL-6 participates in B-cell maturation. Some cytokines are thus involved, directly and indirectly, in both the differentiation and the activation of the various immunocytes, whereas other cytokines inhibit these events to prevent uncontrolled B-cell activation.
There are two important requirements for immune system activation. First, the interaction between receptor molecules and alloantigens is necessary to prime the immunocompetent cells to respond to the cytokines. Second, cytokine release is determined by the type of cellular interactions that take place. Cytokines produced by APC also contribute to the recruitment of host defense cells, even though, in some instances, the overproduction of these cytokines may be harmful to the host. The consequence of the latter include the production of fever, chills, rigors, and increased capillary permeability. These biologic effects may contribute to cellular damage, particularly to damage of capillary endothelial cells by TNF.21,23,24

BIOLGIC EFFECTS OF TRANSFUSED ALLOGENEIC LEUKOCYTES

The total number of leukocytes present in a unit of donor whole blood is approximately 10^8. There is a smaller number in the various blood components, because some are lost during their preparation.25 Table 1 summarizes the approximate total number of leukocytes and lymphocytes in the various cellular blood products available in transfusion practice.

**FNHTR**

An FNHTR is defined as an increase in temperature, of 1°C or more, occurring in association with an allogeneic blood transfusion. FNHTR are estimated to occur in approximately 1% of red blood cell (RBC) transfusions and in approximately 30% of platelet concentrate transfusions.26,27 Most FNHTR have been attributed to the presence, in the recipient’s plasma, of alloantibodies reactive with HLA or other alloantigens present on donor leukocytes and/or platelets.28 Single-donor HLA-unmatched RBC-depleted platelet concentrates are associated with a lower incidence of FNHTR than that seen with pooled random donor platelet concentrates.27,28

The removal of 75% to 90% of the leukocytes from RBC concentrates has been shown to be associated with a significant reduction of the occurrence of FNHTR in most patients.2,26,30,31 Such observations lend credence to the argument that multitransfused patients should receive only leukodepleted blood components to prevent or minimize the severity of such reactions.2,32,36 Although the reduction of the number of allogeneic leukocytes can decrease the overall FNHTR rate compared with that seen with nonleukodepleted products, the use of leukodepleted platelet concentrates has not been totally effective in preventing such reactions.29,37,38 Moreover, it is reasonable to expect that even a pure suspension of platelets could cause FNHTR because platelets are known to have HLA antigens on their surface membranes. However, it has been shown that FNHTR also occur in patients transfused only with the supernatant plasma from platelet concentrates that contain very low numbers of platelets and leukocytes (<10^8 total cells per product).37 In addition, a significantly higher overall transfusion reaction rate has been demonstrated with increasing storage age of both RBC and platelet products.37,39

A correlation between increased levels of TNFα, IL-1β, and IL-6 in platelet concentrates and the frequency of FNHTR has been reported, providing evidence that such reactions may not always be the result of an antigen-antibody event, but may result from the transfusion of soluble biologic mediators, such as cytokines, that are known to be actively synthesized by the leukocytes present in a blood component and that accumulate during storage.38,40 Thus, the removal of leukocytes that actively synthesize and release such cytokines from blood components shortly after collection (presorage leukodepletion) may reduce the incidence of such reactions. Relevantly, it has been shown that the prestorage leukodepletion of platelet concentrates significantly reduces the level of IL-1β, TNF-α, IL-6, and IL-8 in the supernatant plasma.37,39-41 It is also possible that FNHTR in some patients are caused by the release of cytokines from recipient macrophages that might be activated by antigen-antibody-complement complexes formed during or immediately after a transfusion.42 Moreover, it has been suggested recently that cytokines participate in the pathogenesis of some of the signs and symptoms associated with hemolytic transfusion reactions.37,44

Fever is a hallmark of FNHTR. It is likely that the fever is caused by the release of IL-1 and IL-6 by the leukocytes contaminating cellular blood products. There are specific cellular receptors for each cytokine present on the membranes of various cells and it is this cytokine cellular receptor interaction that triggers a specific biologic response. It appears that the febrile response to IL-1 and IL-6 is caused by the production of prostaglandin E2 (PGE2) by cells in the hypothalamus causing the fever. In addition to causing fever, IL-1 can stimulate the recruitment of neutrophils from the bone marrow, a phenomenon that may cause the further elaboration of cytokines to induce other biologic activities. Single-donor HLA-unmatched nonleukodepleted apheresis platelet concentrates associated with a lower incidence of FNHTR than that seen with pooled random donor platelet concentrates.27,28

**Table 1. Approximate Total Leukocyte and Lymphocyte Count of Nonleukodepleted Blood Components Currently Available for Clinical Use**

<table>
<thead>
<tr>
<th>Blood Component</th>
<th>Approximate No. of Leukocytes per Product</th>
<th>Approximate No. of Lymphocytes per Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>10^7</td>
<td>5 x 10^9</td>
</tr>
<tr>
<td>RBC concentrate</td>
<td>5 x 10^8</td>
<td>10^6</td>
</tr>
<tr>
<td>Washed RBC concentrate</td>
<td>10^7</td>
<td>5 x 10^9</td>
</tr>
<tr>
<td>Frozen-deglycerolized RBCs</td>
<td>5 x 10^6</td>
<td>10^6</td>
</tr>
<tr>
<td>Random donor platelet concentrate</td>
<td>5 x 10^7</td>
<td>10^7</td>
</tr>
<tr>
<td>Apheresis single-donor platelet concentrate</td>
<td>5 x 10^8</td>
<td>10^4</td>
</tr>
<tr>
<td>Apheresis single-donor granulocyte concentrate</td>
<td>10^10</td>
<td>5 x 10^9</td>
</tr>
<tr>
<td>Fresh plasma</td>
<td>5 x 10^9</td>
<td>10^3</td>
</tr>
<tr>
<td>Fresh frozen plasma</td>
<td>Undetectable</td>
<td>Undetectable</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>Undetectable</td>
<td>Undetectable</td>
</tr>
</tbody>
</table>

Transfusion-Related Acute Lung Injury (TRALI)

TRALI is a potentially lethal complication of allogeneic blood product transfusions characterized by chills, fever,
blood transfusion. Although the clinical features of severe hypoxemia, occurring 1 to 6 hours after an allogeneic acute respiratory distress, bilateral pulmonary edema, and TRALI thus appears to be an immune-mediated disorder.

Pulmonary vascular leakage associated with TRALI has been shown to be preceded by complement system activation, with neutrophil and endothelial cell injury representing additional factors in the pathogenesis of the acute lung pathology. TRALI has been reported to occur even with the infusion of small quantities of plasma containing leukocyte alloantibodies. It is not clear how the transfusion of only small quantities of plasma can trigger TRALI, but, as the result of such reports, it has been proposed that only the cells of blood donors implicated in such reactions be used for patient care. It has been suggested also that allogeneic blood product units from multiparous donors be screened for HLA or neutrophil-specific antibodies before using such units for transfusion; however, evidence for the efficacy of such a practice is unavailable. The role of the various cytokines in the pathogenesis of TRALI is unclear at the present time.

Platelet Refractoriness and Alloimmunization

Approximately 50% of multiply transfused patients become refractory to pooled random platelet concentrates, limiting the clinical effectiveness of such therapy. Most platelet transfusion refractoriness appears to be caused by alloimmunization to HLA and/or platelet-specific antigens, but the presence of bleeding, concurrent use of drugs, fever, sepsis, disseminated intravascular coagulation, splenomegaly, and ABO blood group incompatibility all may contribute to inadequate allogeneic platelet recovery after transfusion. It has been estimated that HLA and/or platelet-specific alloantibodies can be detected in 20% to 50% of the patients receiving multiple random donor platelets. A recent prospective study showed that HLA antibodies were detected in 13 (26%) of 50 multitransfused patients, with platelet-specific alloantibodies detectable in only 2 and platelet autoantibodies in 2. The exact incidence of platelet alloimmunization causing refractoriness is difficult to ascertain because of the possible presence, in the patients evaluated, of confounding factors that could contribute to inadequate platelet recovery. Moreover, the definition of platelet refractoriness versus that of platelet alloimmunization has been a controversial issue because the detection of alloantibodies does not always parallel the refractory state, and vice versa. In this context, it has been shown that, despite the presence of platelet alloantibodies, some patients continue to have good platelet count increments after allogeneic platelet transfusions.

The precise mechanism of platelet alloimmunization is unknown. Because platelets express only HLA class I antigens, it has been postulated that functional APC bearing both class I and class II HLA antigens, present in donor blood components, are required to initiate an immune response in a recipient. Thus, it has been postulated that APC present antigen peptides in conjunction with donor HLA class II antigens to recipient’s CD4 (T4) cells to induce cytokine production and thus stimulate B cells to produce alloantibodies. Class I HLA peptides may also be recognized by recipient CD8 (T8) T cells, initiating a cytotoxic immune response. This T-cell-mediated immune response thus requires interactions between the APC, the CD4 lymphocyte, and the CD8 T cell.

A variety of methods have been used to reduce or prevent platelet alloimmunization. These include the use of single donor cross-match compatible platelets, the administration of single-donor HLA-matched platelets, UV-B irradiation, and the leukodepletion of allogeneic cellular blood components intended for transfusion. Recent studies indicate that patients receiving leukodepleted random donor blood products are at lower risk for platelet refractoriness than are recipients of nonleukodepleted products. Table 2 summarizes the results of 10 studies comparing the incidence of platelet alloimmunization in patients who had received unmodified blood products with those who had been transfused with leukodepleted blood products. The mean frequency of platelet alloimmunization in patients transfused with nonleukodepleted blood components appears to be considerably higher than that observed in patients who received leukodepleted blood products. Despite these results, many issues remain unresolved concerning the clinical impact of leukodepletion to prevent platelet refractoriness. These include the extent and timing of leukodepletion; the correlation between alloimmunization, refractoriness, and the risk of morbidity due to bleeding; and the cost-effectiveness of such an intervention.

Several investigators have postulated that platelet alloimmunization occurs only with leukocyte counts greater than 10^6 per transfused allogeneic blood component, but the threshold number of leukocytes below which alloimmunization would no longer occur is yet to be determined. Thus, in an experimental animal model, 50% of mice receiving transfusions of platelets with even a very low number of residual leukocytes (≤ 2 × 10^6) became alloimmunized. In another experimental animal model of refractoriness to allogeneic donor platelets, it was shown that 3 log_{10} leukocyte reduction of RBC suspensions provided no significant advantage over 2 log_{10} leukocyte removal, and that approximately 30% of the rabbits that received either 2 or 3 log_{10} leukodepleted allogeneic blood still became refractory. Interestingly, the extent of platelet refractoriness could be reduced by combining plasma removal with leukodepletion. Moreover, it has been shown that the leukodepletion of allogeneic whole blood before its storage (prestorage leukodepletion) was associated with a reduced frequency of platelet refractoriness.
and higher in vivo platelet survival, compared with that seen in animals that received poststorage leukodepleted allogeneic whole blood.79 These experimental animal data suggest that alloimmunization may be related not only to the number of white blood cells present in an allogeneic blood component, but also to the presence of MHC or other antigens, either in the soluble form or as microparticles, that escape leukocyte filtration. Relevantly, it has been reported that the quantity of soluble HLA class I substance present in human whole blood or platelet concentrates does not increase during storage.79 It is probable, therefore, that particulate HLA class I antigens, in the form of microparticles present in allogeneic plasma, contribute to the alloimmunization associated with the transfusion of blood components. The recent demonstration that the combination of leukodepletion and plasma removal abolishes platelet refractoriness supports such an hypothesis, as does the recent report that leukodepletion does not remove membrane microparticles bearing HLA antigens.77,80

Besides the critical role in alloimmunization, the presence of leukocytes in platelet concentrates during storage has been reported to be associated with changes in platelet glycoproteins Ib and IIb/IIIa.81 Such changes may result in the loss of platelet responsiveness to ristocetin and thrombin, but they do not appear to affect in vivo platelet survival.81

Another approach to prevent platelet alloimmunization is the use of UV-B light. Basically, UV-B irradiation appears to modify allogeneic antigen presentation by depressing the degree of expression of both class I and class II HLA antigens on immunocytes. Changes in adhesion molecules such as ICAM-1 and CD14 have been reported with such irradiation interfering with the production of IL-1 and IL-6, which, in turn, might impair antigen presentation by B cells.85 Additionally, UV-B light has been shown to enhance the number of CD8 T-cell population in allogeneic blood product recipients affecting their secondary immune response.85 Most importantly, doses of UV-B that have been shown to reduce the reactivity of mixed lymphocyte reactions do not seem to affect in vitro platelet function or posttransfusion platelet survival.82,83 Studies in dogs that had received UV-B–irradiated allogeneic platelets have been shown to have much lower alloimmunization rate than control animals.84 Relevantly, in two small clinical studies, the rate of platelet alloimmunization was lower in patients transfused with UV-B–treated allogeneic platelet concentrates than in control patients who received nonirradiated platelets.84,85

### Table 2. Summary of Published Studies of Platelet Alloimmunization in Recipients of Either Leukodepleted or Nonleukodepleted Allogeneic Blood Products

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Nonleukodepleted</th>
<th>Leukodepleted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total No. of Patients</td>
<td>No. (%) Alloimmunized</td>
</tr>
<tr>
<td>Ekhollé and Brand86</td>
<td>28</td>
<td>26 (92.8)</td>
</tr>
<tr>
<td>Schiffer et al72</td>
<td>12</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>Fisher et al87</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Murphy et al72</td>
<td>31</td>
<td>15 (48.4)</td>
</tr>
<tr>
<td>Sniecinski et al72</td>
<td>20</td>
<td>10 (50.0)</td>
</tr>
<tr>
<td>Andreu et al74</td>
<td>35</td>
<td>11 (31.4)</td>
</tr>
<tr>
<td>Saarinen et al72</td>
<td>17</td>
<td>12 (70.6)</td>
</tr>
<tr>
<td>van Marwijk Kooy et al73</td>
<td>26</td>
<td>12 (45.1)</td>
</tr>
<tr>
<td>Oksanen et al84</td>
<td>15</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>Handa76</td>
<td>21</td>
<td>8 (38.6)</td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>99/205 (48.3)</td>
</tr>
</tbody>
</table>

Percentages are in parentheses.

* All 24 subjects in this study received leukodepleted blood products. Twelve patients received platelet concentrate transfusions, with a mean leukocyte contamination of 15 × 10^6 per transfusion, whereas the other 12 patients were transfused with platelet concentrates containing less than 5 × 10^6 leukocytes per transfusion. All 5 alloimmunized patients belonged to the first group.

† The mean number of leukocytes per leukodepleted platelet concentrate product transfused was 6 × 10^6.

‡ The leukodepletion of RBC concentrates, pooled platelet concentrates, and single-donor platelet concentrates resulted in 97%, 92%, and 76% leukocyte removal, respectively.

§ The mean leukocyte count after leukodepletion was less than 5 × 10^6 per pooled platelet concentrate consisting of six random donor units.

¶ The mean numbers of residual leukocytes per unit of RBC and platelet concentrate was 0.1 × 10^6 and 0.04 × 10^6, respectively.

### Transfusion-Associated GVHD (TA-GVHD)

TA-GVHD is a potentially lethal immunologically mediated disorder that results from the engraftment of viable immunocompetent allogeneic donor T lymphocytes into the tissues of a blood transfusion recipient.86,87 Whole blood or RBC concentrate transfusions have been implicated in the majority of the cases,25 but granulocyte concentrates,89 platelet concentrates,90 and fresh plasma91 containing viable lymphocytes have also been implicated in TA-GVHD. Frozen blood products have not been reported to cause TA-GVHD.92 The diagnosis of TA-GVHD is usually clinically based, but histopathologic findings of the skin showing lymphocytic infiltration with satellite dyskeratosis may help to distinguish TA-GVHD from drug toxicity or a skin infection. More recently, genetic fingerprinting and the polymerase chain reaction have been used to confirm HLA chimerism in the peripheral blood lymphocytes of affected individuals.93,94

Recently, TA-GVHD has been shown to be caused by a network of interactions involving effector cells, multiple cytokine pathways, and target cells.95 Epithelial and hematopoietic stem cells represent the host target cells, whereas cytotoxic T lymphocytes and natural killer (NK) cells act as the primary effector cells of the allogeneic donor. Although activated NK cells may cause cytolysis by direct cell contact, host tissue damage can occur due to the release of TNF-α, TNF-β, and IL-1 by donor cytotoxic T lymphocytes and NK cells.96 GVHD may be exacerbated by associated herpes or cytokine pathways, and target cells.96,97 Allogeneic donor T lymphocytes into the tissues of a blood transfusion recipient.86,87 Whole blood or RBC concentrate transfusions have been implicated in the majority of the cases,25 but granulocyte concentrates,89 platelet concentrates,90 and fresh plasma91 containing viable lymphocytes have also been implicated in TA-GVHD. Frozen blood products have not been reported to cause TA-GVHD.92 The diagnosis of TA-GVHD is usually clinically based, but histopathologic findings of the skin showing lymphocytic infiltration with satellite dyskeratosis may help to distinguish TA-GVHD from drug toxicity or a skin infection. More recently, genetic fingerprinting and the polymerase chain reaction have been used to confirm HLA chimerism in the peripheral blood lymphocytes of affected individuals.93,94

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The occurrence of a TA-GVHD in a blood transfusion recipient depends on the immunocompetence of the host, the genetic similarity between donor and recipient, and the number of viable donor T lymphocytes present in the transfused blood product. The precise number of transfused T lymphocytes needed to induce TA-GVHD is not known. Data from animal studies suggest that 10^7 lymphocytes per kilogram of body weight may be required. In humans, as few as 10^4 lymphocytes administered to children with severe combined immunodeficiency has resulted in TA-GVHD.

Typically, TA-GVHD has been reported to occur in immunosuppressed patients whose immune systems are impaired as a result of prematurity, a congenital immunodeficiency state, a hematologic malignancy, a solid tumor, or associated with bone marrow transplantation. Evidence indicates that TA-GVHD can also affect immunocompetent individuals. The risk of the development of TA-GVHD in nonimmunocompromised individuals appears to be greater if there is some HLA-identity between donor and recipient such as occurs with transfusions from first-degree relatives. The likelihood that blood from unrelated homozygous donors will be transfused into heterozygous patients is greater in a monoracial population and has been estimated to be 1 in 16,835 in France, 1 in 650 in Japan, and 1 in 7,174 in American whites.

The prevention of TA-GVHD can be accomplished by altering either the viability or the total number of T lymphocytes in a cellular blood product before transfusion. γ-Irradiation is the procedure generally used to prevent TA-GVHD. The recommended minimum dose of 15 Gy has been shown to decrease the lymphocyte mitogen response by 90% without compromising the function of the other blood cells. Although higher doses of γ-irradiation, such as 500 Gy, may affect granulocyte chemotaxis and bactericidal activity, granulocyte function remains essentially normal after γ-irradiation ranging from 0 to 175 Gy. The γ-irradiation of blood products does not appear to cause significant adverse effect on stored blood cells; nevertheless, some investigators recommend that γ-irradiated RBC concentrates, used for intratracheal or pediatric transfusions, be washed to reduce the elevated plasma potassium levels often seen in such units. Furthermore, irradiated RBC concentrates have been shown to have minor changes in ATP, pH, lactate dehydrogenase (LDH), and plasma-free hemoglobin levels after 42 days of storage when compared with non-γ-irradiated RBC units. Nonetheless, the routine washing of irradiated RBC before transfusion does not appear to be warranted.

A survey conducted in 1989 showed that various institutions associated with the American Association of Blood Banks used the following γ-irradiation doses: 15 Gy (42.3%), 15 to 25 Gy (31.8%), 25 to 35 Gy (22.8%), and 25 to 50 Gy (3.1%). Because a small percentage of lymphocytes may survive 15 Gy irradiation, it has been suggested that doses of 25 to 30 Gy of γ-irradiation may be appropriate to prevent TA-GVHD. The FDA in the United States has recently established regulations regarding γ-irradiation doses of blood products intended for clinical use. These guidelines require 2,500 cGy (25 Gy) to the central part of the container, with 1,500 cGy (15 Gy) as the minimum dose to any other point.

Alternative approaches to prevent TA-GVHD have been reported. In animal studies, the UV-irradiation of transfused leukocytes has been shown to abolish TA-GVHD in recipient dogs. Another approach has been to reduce the number of donor lymphocytes using leukocyte filters that provide at least 3 log_10 removal. In vitro studies indicate that leukodepleted cellular blood components may be associated with decreased risk for TA-GVHD; however, TA-GVHD has been reported to occur even with the transfusion of leukodepleted blood components (99% to 99.6% leukocyte removal).

Immunomodulation

Blood transfusion and allograft transplantation. The beneficial effect of allogeneic blood transfusions (ABT) in renal transplantation was described more than 20 years ago. A highly significant correlation was reported between increased numbers of pretransplant blood transfusions and improved allograft survival in a prospective analysis of 1,360 kidney transplants. Over the past 20 years, these results have been replicated in animal experiments and confirmed clinically in many transplant centers. Overall, patients transfused with allogeneic blood products have a significantly better renal allograft survival rate than nontransfused patients, regardless of the number of HLA-A locus and HLA-B locus mismatches. Similar data are also available for HLA-DR mismatching. Even with HLA-identical sibling allografts, it has been reported that 33% of allogeneically transfused recipients experience graft rejection, compared with 75% of untransfused recipients. Thus, although data exist to indicate that the beneficial effect of ABT occurs even in HLA-identical recipients, the ABT effect is most evident when the degree of HLA mismatching is greatest.

Although it is generally accepted that ABT can improve renal allograft survival, the influence of such transfusions has declined significantly over the last decade, probably caused by the advent of very effective immunosuppressive drugs and improved patient management, particularly the treatment of rejection episodes. However, a recent multicenter study reporting on 58,036 renal transplants since the advent of the use of cyclosporine indicates that patients receiving ABT are still more likely to have successful renal allografts than those that did not. Similar results have been observed in patients receiving living-related-donor kidney transplants. The mechanism involved in producing the ABT-associated prolonged allograft survival has not yet been completely elucidated. In addition, there are many unanswered questions about the use of ABT in such patients, such as the optimal number of transfusions, the component of blood required to produce the effect, the volume of blood required with each transfusion, the optimal timing of the transfusions to produce the effect, the concurrent hazards of the blood transfusion induced immunosuppressive effect, and whether ABT are still necessary.

It has been proposed that the ABT effect may occur because of the selection of nonresponders, the development of a suppressor cell network, the inactivation of alloreactive
clones by immunosuppression, and/or the induction of anti-idiotypic and anticonlonotypic antibodies.\textsuperscript{53,129} The exclusion of responders in the cross-match test cannot account for the beneficial effect, because ABT have been shown to improve renal allograft survival even in HLA-identical donor-recipient combinations.\textsuperscript{131} Similarly, the clonal deletion theory proposed by Terasaki is no longer supported.\textsuperscript{129,132} There is considerable evidence to suggest that ABT induce the production of suppressor T cells; however, this effect does not seem to persist.\textsuperscript{131} Anti-idiotypic antibodies, which are predominantly of the \textit{V}\textsubscript{\textalpha} gene family, have been shown to be present in serum of ABT recipients and in patients with long-term functioning allografts.\textsuperscript{133} Furthermore, the presence of an anti-Fc receptor antibody in the serum of kidney recipients has been associated with an allograft survival rate of 93\% at 1 year and 71\% at 3 years, compared with 29\% at 1 year and 25\% at 3 years in patients lacking such antibodies.\textsuperscript{134}

It has been suggested that the beneficial effect of ABT on renal allograft survival increases with the number of transfusions. Thus, patients who received 6 to 10 transfusions have been reported to have higher 1-year graft survival rates than patients who had received fewer allogeneic transfusions. Interestingly, patients who receive more than 10 transfusions appear to have lower overall graft survival rates.\textsuperscript{135} This may be caused by the greater likelihood that multitransfused patients will develop cytotoxic antibodies and, thus, are at greater risk for earlier and more severe allograft rejection episodes.\textsuperscript{130} Relevantly, recipients of whole blood or RBC concentrates have been shown to have better 1-year cadaveric graft survival than individuals receiving leukocyte-poor blood components such as washed RBC concentrates or frozen-deglycerolized RBCs. Such data indicate that allogeneic donor leukocytes somehow participate in the production of the beneficial ABT effect.\textsuperscript{136} Thus, pretransplant ABT remain an intervention that can be useful in the management of selected patients scheduled for kidney transplantation. Patients transfused during surgery had no impact on subsequent renal allograft survival.\textsuperscript{125} Nonetheless, additional data are required to understand how ABT induce their beneficial effect in renal transplantation. In addition, the potential hazards of the ABT should be considered before being undertaken in a particular patient.

**Blood transfusion and tumor growth.** The possible association between ABT and cancer recurrence was first suggested more than 10 years ago, when concern was raised for patients undergoing curative surgery for a malignant disorder who might be adversely affected by the immunomodulatory effects of ABT administered perioperatively.\textsuperscript{137} No definitive data proving this effect in humans have been published as yet. What have been reported are mainly nonrandomized retrospective clinical data. Many reports have appeared evaluating the effect of perioperative ABT on tumor recurrence and/or overall prognosis in patients with a malignancy undergoing curative cancer surgery. A considerable amount of such data has been obtained from studies of patients with colorectal carcinoma (summarized in Table 3).\textsuperscript{138-162} Evidence for a deleterious ABT effect has also been provided by more than 40 clinical studies in patients with a variety of other malignancies, including breast, lung, kidney, prostate, stomach, cervix, vulva, head and neck, larynx, soft tissue, bone, and liver metastases. The adverse effect of ABT has thus been reported in approximately 50\% of such studies, with the remaining 50\% reporting no adverse effect.\textsuperscript{168}

The most relevant clinical studies evaluating the effect of perioperative ABT on the outcome in patients with colorectal carcinoma have been subjected recently to meta-analysis by two groups of investigators.\textsuperscript{166,167} One such analysis indicates that the cumulative odds ratio of colorectal carcinoma recurrence, cancer-associated death, and death from any cause in allogeneically transfused patients are 1.80, 1.76, and 1.63, respectively.\textsuperscript{166} These investigators concluded that their analysis supports the hypothesis that perioperative ABT are associated with an increased risk of colorectal carcinoma recurrence and death from this disease.\textsuperscript{169} The second group concluded that the adverse effect of ABT might increase the relative risk of cancer recurrence by 37\% (95\% confidence index [CI], 20\% to 56\%).\textsuperscript{170} Interestingly, a recently published prospective randomized study in colorectal cancer patients concluded that the risk of cancer recurrence was increased in patients transfused with either allogeneic or syngeneic blood compared with untransfused subjects.\textsuperscript{165} In contrast, a similar prospective randomized study concluded that ABT in colorectal carcinoma patients were an independent prognostic factor on the cancer recurrence rate.\textsuperscript{165} Thus, whether ABT affect tumor growth, in humans, is still an open issue.

The tumor growth-promoting effect of ABT has been shown to occur in experimental animals.\textsuperscript{171-175} Data from both inbred and outbred animal models indicate that the tumor growth-promoting effect of ABT is mediated immunologically; as the ABT tumor growth-promoting effect can be adoptively transferred, using spleen cells, to naive animals.\textsuperscript{171} These studies indicate that the ABT effect on the growth of animal tumors is related to the presence of donor leukocytes in the allogeneic blood, and that this deleterious effect can be ameliorated by the prestorage leukodepletion of the allogeneic blood.\textsuperscript{173,175} Moreover, the data provide evidence for the lack of efficacy of poststorage leukodepletion in preventing the ABT tumor growth promotion effect.\textsuperscript{175} Although results from experimental animals cannot necessarily be extrapolated to the clinical situation, the results obtained indicate that the bedside (poststorage) leukodepletion of allogeneic blood products may not be effective in preventing the tumor growth-promoting effect of ABT, if this effect indeed occurs in humans.

**Blood transfusion and infection.** ABT are administered frequently to patients who are at risk for infections. The association between perioperative ABT and the increased incidence of bacterial infections after surgery has been examined in both clinical and experimental animal studies. The available clinical data are mainly from uncontrolled studies. The results of 9 relevant studies are summarized in Table 4.\textsuperscript{176-184} The only prospective trial conducted in colorectal cancer patients, in which subjects were randomly assigned syngeneic or allogeneic blood, reported a significant higher postoperative infection rate in patients transfused with allogeneic blood compared with patients who received syngeneic
Table 3. Summary of 30 Clinical Studies That Analyzed the Effect of Perioperative Allogeneic Blood Transfusions on Clinical Outcome (Recurrence Rate and/or Overall Prognosis) in Patients With Colorectal Carcinoma

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Study Design</th>
<th>No. of Patients</th>
<th>Adverse Effect of ABT on Outcome (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burrows and Tarter</td>
<td>Retrospective</td>
<td>123, 58, 65</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Nathason et al</td>
<td>Retrospective</td>
<td>366, 199, 167</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Blumberg et al</td>
<td>Retrospective</td>
<td>197, 129, 68</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Foster et al</td>
<td>Retrospective</td>
<td>146, 65, 81</td>
<td>.03</td>
</tr>
<tr>
<td>Frankish et al</td>
<td>Prospective, not randomized</td>
<td>174, 103, 71</td>
<td>NS</td>
</tr>
<tr>
<td>Ota et al</td>
<td>Retrospective</td>
<td>207, 162, 45</td>
<td>NS</td>
</tr>
<tr>
<td>Parrot et al</td>
<td>Retrospective</td>
<td>517, 373, 144</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Burrows et al</td>
<td>Retrospective</td>
<td>295, 177, 118</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Francis and Judson</td>
<td>Retrospective</td>
<td>87, 53, 34</td>
<td>NS</td>
</tr>
<tr>
<td>Ross et al</td>
<td>Retrospective</td>
<td>159, 95, 64</td>
<td>NS</td>
</tr>
<tr>
<td>Voogt et al</td>
<td>Retrospective</td>
<td>113, 86, 27</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Weidem et al</td>
<td>Retrospective</td>
<td>171, 103, 68</td>
<td>.01*</td>
</tr>
<tr>
<td>Cormen et al</td>
<td>Retrospective</td>
<td>400, 343, 57</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>van Lawick van Pabst et al</td>
<td>Retrospective</td>
<td>164, 117, 47</td>
<td>.039</td>
</tr>
<tr>
<td>Beyon et al</td>
<td>Retrospective</td>
<td>519, 385, 134</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Crowsen et al</td>
<td>Retrospective</td>
<td>525, 373, 152</td>
<td>NS</td>
</tr>
<tr>
<td>Mecklin et al</td>
<td>Retrospective</td>
<td>520, 355, 165</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Vente et al</td>
<td>Retrospective</td>
<td>212, 158, 54</td>
<td>NS</td>
</tr>
<tr>
<td>Waymack et al</td>
<td>Retrospective</td>
<td>155, 101, 54</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Wobbes et al</td>
<td>Retrospective</td>
<td>270, 184, 86</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cheshyn-Curtis et al</td>
<td>Retrospective</td>
<td>961, 591, 370</td>
<td>NS</td>
</tr>
<tr>
<td>Jakobsen et al</td>
<td>Retrospective</td>
<td>315, 268, 47</td>
<td>NS</td>
</tr>
<tr>
<td>Liewald et al</td>
<td>Retrospective</td>
<td>439, 304, 135</td>
<td>.02</td>
</tr>
<tr>
<td>Marsh et al</td>
<td>Retrospective</td>
<td>132, 62, 70</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Quintillan et al</td>
<td>Prospective, not randomized</td>
<td>58, 35, 23</td>
<td>NS</td>
</tr>
<tr>
<td>Heiss et al</td>
<td>Prospective Randomized</td>
<td>120, NA, NA</td>
<td>.008</td>
</tr>
<tr>
<td>Tarter</td>
<td>Prospective, not randomized</td>
<td>339, 110, 229</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Sene et al</td>
<td>Prospective, not randomized</td>
<td>379, 221, 158</td>
<td>NS</td>
</tr>
<tr>
<td>Tang et al</td>
<td>Retrospective</td>
<td>725, 472, 253</td>
<td>NS</td>
</tr>
<tr>
<td>Busch et al</td>
<td>Prospective, randomized</td>
<td>475, 236, 239†</td>
<td>.01</td>
</tr>
</tbody>
</table>

Total                         9,263, 5,918, 3,225

Abbreviations: NS, not significant; NA, not available.
* Transfused patients had a lower tumor recurrence rate than untransfused patients.
† Patients in the control group received syngeneic (autologous) blood transfusions.

blood transfusions (27% vs 12%, P = .036). Using multivariate regression analysis to adjust for other factors, the odds ratio for risk of postoperative infections in the allogeneic versus syngeneic blood transfusion group was 2.84 (95% CI, 1.02 to 7.98). The observed number of noninfectious complications was similar in the two groups of patients.184

The dose-response relationship between the number of allogeneic blood units transfused and the risk of infection has also been examined. Thus, in a study of 137 patients who underwent surgery for a penetrating colonic injury, the risk of an infection was 7.5% for untransfused patients, and 25%, 37%, and 57% for patients transfused with 1 to 5 units, 6 to 9 units, or 10 or more units, respectively.185 In a retrospective study of patients who underwent surgery for gastric carcinoma, patients who developed postoperative infections were transfused with a higher number of allogeneic units compared with those who did not. However, infected patients were found to have been subjected to longer surgery, more frequent use of urinary tract catheters and drains, and had an operation involving a resection.186

The role of allogeneic leukocytes in the development of postoperative infections has also been investigated. A prospective randomized trial involving patients who underwent colorectal surgery has shown that patients transfused with allogeneic whole blood had a significantly higher frequency of postoperative infections than patients who received 99.98% leukodepleted allogeneic blood. This study also provided evidence that NK cell function remained significantly impaired for up to 30 days after surgery in those patients transfused with nonleukodepleted allogeneic whole blood.
partially because of the problem of defining the term "infec-
tion." Limiting the definition of infection to positive cultures underestimates prevalence, whereas extending the definition to include fever overestimates prevalence. This is the opinion, therefore, that a large prospective randomized clinical trial is required to validate definitively the association between ABT and increased risk of infection.

Blood transfusion and spontaneous recurrent abortions. Because the fetus represents a semiallogeneic graft to its mother, the maintenance of a pregnancy is dependent on immunologic equilibrium between the implanted fetus and the maternal immune response to the fetus. It has been proposed that an idiotypic–anti-idiotypic network downregulates maternal rejection during pregnancy. 189,190 This delicate balance may be altered in situations in which the spouses share HLA antigens. In such situations, maternal blocking antibodies may not form and the implanted fetus is rejected. Based on such an hypothesis and also on evidence of prolonged graft survival seen in allogeneically transfused kidney transplant recipients, leukocyte transfusions have been used as a form of immunotherapy to treat women with spontaneous recurrent abortions. 191 Different allogeneic leukocyte transfusion protocols have been used with leukocytes from either sexual partners or third-party donors. Such allogeneic leukocytes have been transfused as pooled buffy-coats; as single donor buffy-coats; as RBC suspensions containing leukocytes; or intravenously, intracutaneously, or as an intradermal injection of mononuclear cells obtained by gradient separation. 192

The success rate of approximately 75% obtained with the transfusion of either paternal or third-party leukocytes is considerably better than that observed with maternal cells of approximately 50% (Table 5). 191,192 The latter figure is derived from three prospective nonrandomized studies that...


Table 5. Summary of the Clinical Outcome of Patients With a History of Recurrent Spontaneous Abortions who Received Leukocyte Transfusions

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Paternal Lymphocytes</th>
<th>Third-Party Donors</th>
<th>Maternal Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taylor and Faulk191</td>
<td>0/10 (0.0)</td>
<td>3/4 (75.0)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>Mowbray et al235</td>
<td>17/22 (77.3)</td>
<td>10/27 (37.0)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>McIntyre et al104</td>
<td>0/10 (0.0)</td>
<td>23/26 (88.5)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>Mowbray et al136</td>
<td>24/30 (80.0)</td>
<td>0/10 (0.0)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>Reznikoff-Ethirant et al196</td>
<td>29/34 (85.3)</td>
<td>0/10 (0.0)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>Smith and Cowchock197</td>
<td>43/74 (58.1)</td>
<td>0/10 (0.0)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>Takakuwa et al198</td>
<td>28/35 (80.0)</td>
<td>0/10 (0.0)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>Carp et al199</td>
<td>64/89 (71.9)</td>
<td>0/10 (0.0)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>Cauchi et al200</td>
<td>13/21 (61.9)</td>
<td>0/10 (0.0)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>Ho et al201</td>
<td>31/39 (79.5)</td>
<td>8/11 (72.7)</td>
<td>32/49 (65.3)</td>
</tr>
<tr>
<td>Unander202</td>
<td>24/26 (92.4)</td>
<td>0/10 (0.0)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>Gatenby et al203</td>
<td>13/19 (68.4)</td>
<td>9/19 (47.4)</td>
<td>0/10 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>262/363 (72.2)</td>
<td>277/304 (91.1)</td>
<td>51/95 (53.7)</td>
</tr>
</tbody>
</table>

Percentages are in parentheses.

compared patients who received either paternal or maternal leukocytes.193,201,202 The number of allogeneic mononuclear cells inoculated may also influence the outcome. The infusion of less than 60 million mononuclear cells may result in a suboptimal effect, whereas the inoculation of excessive cells may not be effective.203,204 The use of allogeneic leukocytes in the treatment of patients with spontaneous recurrent abortions is of course associated with the same risks associated with any other leukocyte transfusion, including neonatal GVHD. Moreover, allogeneic leukocytes may cause fetal growth retardation, congenital anomalies, and alloimmune neonatal neutropenia.192,203,205

The mechanism of the beneficial action of the allogeneic leukocyte transfusions in patients with spontaneous recurrent abortions is unknown. However, it has been suggested that the beneficial clinical response observed may be associated with the production of auto-antidiotypic antibodies,206 release of IL-1,207 or decrease in maternal IL-2 receptor level.208

The efficacy of leukocyte transfusions in patients with spontaneous recurrent abortions remains to be established by proper prospective randomized studies. Limitations to data currently available include small numbers of women in some studies; heterogeneity of the study populations; great diversity of the therapy protocol used; and possible intervention effects derived from placebo treatments used in some of the control groups of patients.203,209 Only well-designed randomized cooperative studies will clarify the possible true benefit, risks, and clinical indications for allogeneic leukocyte transfusions in the treatment of women with spontaneous recurrent abortions.

Blood transfusion and inflammatory bowel disease. Crohn’s disease is a chronic inflammatory condition that can affect both the small and large bowel to cause clinical symptoms of diarrhea and crampy abdominal pain. Typically, after many years of quiescent periods alternating with periods of considerable disease activity, patients with Crohn’s disease require surgical treatment because of a bowel obstruction or perforation. The recurrence rate after surgical resection is approximately 50% at 10 years.210 Although the etiology of the Crohn’s disease is still uncertain, it has been suggested that immunologic mechanisms are involved in its pathogenesis. In 1986, Tartert et al211 reported that patients with Crohn’s disease had a lower number of circulating total lymphocytes and T lymphocytes compared with normal controls, and that multiple ABT were associated with a significantly lower peripheral total lymphocyte and T-cell counts after surgery. Postulating that the immunosuppressive effects of allogeneic blood might be beneficial to patients with Crohn’s disease, several studies examined whether the postoperative recurrence rate is affected by the perioperative use of ABT. The pooled data from 5 available studies suggest that the recurrence rate in the two groups is similar (Table 6).212,216 However, the studies summarized in Table 6 are difficult to compare because they have different follow-up periods and use different surgical treatments. Most importantly, they are all retrospective.

The effect of ABT on the number of postoperative septic complications in patients with Crohn’s disease has also been analyzed. In 1988, Tartert,217 using multivariate analysis, implicated ABT as a major factor in the development of infectious complications in Crohn’s disease patients subjected to surgical procedures. Such an association was not shown to reach statistical significance in another similar study.213 Because many factors might affect recurrent rates of Crohn’s disease, as well as the rate of septic complications after resection, it will be necessary to conduct well-designed prospective trials to clarify whether ABT have an impact in the disease activity of patients with Crohn’s disease.

THE MECHANISM OF THE ABT-INDUCED IMMUNOMODULATION

From the available evidence, it appears that ABT-induced immunomodulation is caused by the presence of contaminating leukocytes in the transfused cellular blood products.125,127,173,175 The mechanism of the immunomodulation has not yet been precisely elucidated; however, it has been postulated that the immunosuppressive effects are immunologically mediated.55,127,218 Allogeneic leukocytes present in cellular blood products bear class II major histocompatibility complex (MHC) antigens and it has been suggested that donor APCs present these allogeneic MHC antigens to the T lymphocytes of the recipient.212 This MHC antigen–T lymphocyte interaction provides the first signal that culminates in the expression of the IL-2 receptor. This is insufficient to elicit an immune response, because a second signal is required to induce the secretion of the various cytokines, which, in turn, cause the proliferation and differentiation of alloantigen-specific T lymphocytes.219

The immunogenicity of MHC antigens present on allogeneic blood products thus depends on the ability of donor APCs to stimulate recipient T cells by delivering two different signals. The absence of the second signal has been shown to induce T-cell anergy.206 In this context, it has been suggested recently that, during storage, leukocytes lose their
Thus, it might be possible to reduce the risk of TA-CMV infection from asymptomatic seropositive donors by limiting the number of blood units transfused to subgroups of patients at high risk, and/or improving the ability to detect IgM anti-CMV to identify acutely infected blood donors who may be more infectious than chronic carriers. CMV has been isolated from the peripheral blood leukocytes of many infected individuals. Moreover, individuals transfused with granulocyte concentrates have been shown to be at increased risk for CMV infection. Thus, it has been suggested that the removal of leukocytes from allogeneic donor blood may prevent TA-CMV. In this context, the use of frozen-deglycerolized RBC has been reported to prevent the transmission of CMV and the transfusion of washed RBC (87% leukocyte removal) from CMV-seronegative donors to neonates is associated with a lower frequency of anti-CMV seroconversion (1.3%) than that seen in recipients of unwashed RBC (13% to 35%).

The maintenance of a satisfactory inventory of CMV-seronegative blood donors may be difficult for some centers. The use of leukocyte filters in preventing TA-CMV infection has therefore been the subject of several studies. In a multicenter controlled trial, 21% of newborn infants transfused with unfiltered blood products developed CMV infection, whereas no TA-CMV was observed in the group of infants transfused with leukodepleted blood products (P = .005). In one retrospective study, none of 59 CMV-seronegative patients with acute leukemia or non-Hodgkin’s lymphoma had evidence of seroconversion 2 months after transfusion with leukodepleted blood products. Another retrospective study reported that only 2 of 13 CMV-seronegative patients who were transfused with lymphocyte depleted allogeneic bone marrow from CMV-positive donors, and who were transfused with leukodepleted CMV-unscreened blood products, developed clinical manifestations of CMV infection. It has been reported that leukocyte filters are also capable of removing CMV-DNA from CMV-infected donor blood. The data from a prospective randomized study in 487 patients undergoing bone marrow transplanta-

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Study Design (time period)</th>
<th>No. of Patients</th>
<th>Recurrence Rate</th>
<th>Statistical Significance (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Williams and Hughes</td>
<td>Retrospective (1973-1986)</td>
<td>60*</td>
<td>4/28</td>
<td>15/32</td>
</tr>
<tr>
<td>Peters et al</td>
<td>Retrospective (1976-1985)</td>
<td>79†</td>
<td>10/45</td>
<td>15/34</td>
</tr>
<tr>
<td>Sutherland et al</td>
<td>Retrospective (not cited)</td>
<td>88</td>
<td>12/26</td>
<td>13/42</td>
</tr>
<tr>
<td>Scott et al</td>
<td>Retrospective (1947-1988)</td>
<td>197‡</td>
<td>38/102</td>
<td>35/95</td>
</tr>
<tr>
<td>Steup et al</td>
<td>Retrospective (1980-1985)</td>
<td>104§</td>
<td>30/65</td>
<td>20/39</td>
</tr>
<tr>
<td>Total</td>
<td>—</td>
<td>508</td>
<td>100/266</td>
<td>98/242</td>
</tr>
</tbody>
</table>

* Both groups were similar in age, duration of Crohn’s disease, and serum albumin level. They had different hemoglobin levels, length of bowel resected, and location of small bowel involvement.
† Both groups were similar in age, sex, race, and duration of disease. The multitransfused group had significantly lower hematocrit and albumin levels on admission. The untransfused group included patients receiving 1 unit of blood.
‡ There were significant differences in the hemoglobin and albumin levels between the two groups. The distribution of ABO and Rhesus blood groups was similar in the two groups.
§ The transfused group had significantly lower hemoglobin levels and longer bowel segments resected.

LEUKOCYTES AS THE VECTOR FOR TRANSFUSION-ASSOCIATED INFECTIOUS DISEASES

Cytomegalovirus (CMV) Infections

Transfusion-associated CMV (TA-CMV) infections have been recognized as a significant cause of morbidity and mortality in blood product recipients at risk. These include CMV-seronegative pregnant women, premature infants born to CMV-seronegative mothers, CMV-seronegative allogeneic bone marrow transplant recipients, and CMV-seronegative patients with acquired immunodeficiency syndrome (AIDS). CMV antibody prevalence rates in North America range from 30% to 80%. Whether all seropositive blood donors can transmit CMV is unknown, and recipient factors may be critical in determining the development of TA-CMV. Thus, it might be possible to reduce the risk of TA-CMV infection from asymptomatic seropositive donors by limiting the number of blood units transfused to subgroups of patients at high risk, and/or improving the ability to detect IgM anti-CMV to identify acutely infected blood donors who may be more infectious than chronic carriers. CMV has been isolated from the peripheral blood leukocytes of many infected individuals. Moreover, individuals transfused with granulocyte concentrates have been shown to be at increased risk for CMV infection. Thus, it has been suggested that the removal of leukocytes from allogeneic donor blood may prevent TA-CMV. In this context, the use of frozen-deglycerolized RBC has been reported to prevent the transmission of CMV and the transfusion of washed RBC (87% leukocyte removal) from CMV-seronegative donors to neonates is associated with a lower frequency of anti-CMV seroconversion (1.3%) than that seen in recipients of unwashed RBC (13% to 35%).

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BIOLOGIC EFFECTS OF LEUKOCYTES

The leukodepletion of the blood products can be performed shortly after collection (prestorage leukodepletion) or after storage, just before transfusion (poststorage leukode-}

tion indicate that the use of leukodepleted blood products is equivalent to the use of CMV-seronegative blood products in preventing TA-CMV infection.244

Other Infections

Leukocytes, particularly B and T lymphocytes, are also the reservoir of other infectious agents that can be transmitted by blood transfusion. These include the human immunodeficiency viruses (HIV-1 and HIV-2), the human T-cell leukemia viruses (HTLV-I and HTLV-II), the Epstein-Barr virus (EBV), as well as the hepatitis C virus (HCV).87,235 However, the minimum number of leukocytes capable of transmitting such infections has not yet been determined. In this context, it has been shown that the leukocyte filtration process can remove intact HIV-1-infected cells and infectious particulate debris but not free virus.236,237 Interestingly, allogeneic peripheral blood mononuclear cells have recently been shown to induce the activation of HIV-1-infected cells and the subsequent dissemination of HIV-1 to uninfected cells. This in vitro effect was not observed with leukodepleted allogeneic blood.238 Additionally, a recently published retrospective study has shown an increased incidence of opportunistic infections in previously transfused HIV-1-infected patients.239 Such findings could have important implications in clinical decision-making regarding the use of leukodepleted blood products in HIV-infected individuals.

Although leukodepletion is not sufficient to completely remove HTLV-I from infected blood, it has been suggested that the transmission of HTLV-I to can be prevented, at least partially, by donor blood leukodepletion.240,241 Additionally, it has been shown that the infectivity of HTLV-positive blood products decreases with storage, probably because of the death of infected lymphocytes at refrigerator temperature.87 Experimental data indicate that only 10% of HTLV-I-positive units stored for 20 days were virus culture-positive, whereas all units stored for less than 7 days were still positive.242

Although EBV has not often been reported as a transfusion-associated infection, this B-cell-associated virus can be transmitted by ABT causing an infectious mononucleosis type of syndrome.243 EBV can also be transmitted to transplant recipients by transfused allogeneic leukocytes, thus exposing them to the potential risk of a lymphoproliferative disorder.244,245 The removal of leukocytes from blood products thus might prevent EBV transmission; however, no evidence has yet been provided to support this hypothesis.

The bacterial contamination of the blood products remains a significant problem in transfusion medicine.246-248 For the past 13 years, the annual incidence of positive bacterial cultures in blood components tested by the Canadian Red Cross Society has been approximately 0.3%.247,248 Moreover, posttransfusion septic reactions account for approximately 10% of the transfusion-associated deaths reported in the United States.249 Possible measures to minimize transfusion-associated sepsis include improved donor screening, reduction in blood product storage periods, improved techniques for blood collecting and processing, quality control programs, bacteriologic screening, and the removal of contaminating bacteria.247-250 Recently, the removal of bacteria contaminating blood products has been demonstrated by leukodepletion. It appears that removal of leukocytes from blood products that have phagocyted bacteria lowers the risk of transfusion-associated septic reactions.251-254 However, the removal of leukocytes should be performed only after an appropriate incubation period to enable the phagocytosis of the bacteria present.253,254 Leukocyte depletion filters have also been reported to be able to remove contaminating uningested bacteria that may be present in blood components directly.249,255,256 The relative contribution of the phagocytic mechanism versus the direct mechanism in the removal of bacteria from blood products has not yet been delineated.

THE LEUKOCYTE DEPLETION OF BLOOD PRODUCTS

Leukocyte Depletion of Blood Products

The removal of leukocytes from blood products can be accomplished by various techniques including sedimentation, centrifugation, washing, freezing-thawing, and leukofiltration.256 Recently, a number of articles have provided information about the filtration of blood products including leukocyte depletion.1,2,4,5,3,257-259 First-generation blood filters (170 to 240 μm) are intended to prevent the infusion of large clots and large cellular aggregates to blood product recipients; second-generation filters (40 μm), also called microaggregate filters, remove cellular microaggregates that often form during blood storage; and third-generation blood filters can retain not only microaggregates but also cells and are generally referred to as leukocyte depletion filters.258 Significant progress has been made over the past decade in the biotechnology of leukofiltration. Thus, donor leukocytes can be removed by both physical (barrier retention, surface phenomena, and charge density) and biologic (cell adhesion and cell-cell interaction) mechanisms. For an excellent discussion about what is known currently about leukofiltration, the interested reader is referred to the report by Dzik.259

Currently available leukocyte filters provide depletion rates that exceed 3 log10 (99.9%), resulting in less than 3 × 106 leukocytes in a 300-mL unit of a blood component, ie, approximately 10 residual leukocytes per microliter. Various techniques have been devised to count accurately such low levels of residual leukocytes in leukodepleted blood products, because traditional cell counting techniques are inaccurate at this low number.260 The most widely accepted current method uses a counting chamber known as the Nageotte chamber. The Nageotte chamber is a large-volume hemocytometer that uses a sample volume of 50 μL. This approach provides accurate detection of 0.5 to 1 leukocytes per microliter. This corresponds to approximately 3 × 105 residual leukocytes in a 300-mL unit of RBCs.261,262 Flow cytometry techniques have been reported to have a detection sensitivity to approximately 0.1 leukocytes/μL,263 and to have less variability than the Nageotte chamber.260,262,263 However, flow cytometry requires expensive equipment and reagents, and may not be available in many centers requiring such technology.

The leukodepletion of the blood products can be performed shortly after collection (prestorage leukodepletion) or after storage, just before transfusion (poststorage leukode-
The prestorage leukodepletion of blood products has been shown to prevent some of the adverse effects caused by metabolites released from leukocytes during blood product storage. Moreover, the prestorage leukodepletion of blood products has been shown to be more effective than poststorage leukodepletion in preventing platelet alloimmunization and the tumor-growth promoting effect associated with ABT. The latter observations are from studies in experimental animals and need to be confirmed in humans.

Clinical Indications for Using Leukodepleted Blood Products

The proposed clinical indications for the use of leukodepleted blood products have been reviewed in several recent publications. These include the prevention of FNHTR, GVHD, the transmission of disease, refractoriness to transfused platelets, and immunomodulation. However, in most of these, the effectiveness of leukodepletion remains unproven. Moreover, the exact degree of leukodepletion required for the various proposed clinical situations remains to be defined. Thus, it has been suggested that 1 log₁₀ leukocyte reduction prevents FNHTR; that a 2 log₁₀ reduction may prevent the transmission of viruses; and that a ≥ 3 log₁₀ reduction may be necessary to prevent platelet alloimmunization. However, because there are no data available as guidelines for the use of leukodepleted blood products for most clinical indications, the use of leukocyte filters should be restricted to selected patients for whom such data exist. Despite the latter, it has been suggested recently that the use of leukodepleted blood components might be a very cost-effective strategy to prevent platelet alloimmunization in adult patients with acute leukemia. In our opinion, further data are required before the routine implementation of leukodepletion for all blood products to prevent platelet alloimmunization as well as other indications.

SUMMARY

A considerable literature has accumulated over the past decade indicating that leukocytes present in allogeneic cellular blood components, intended for transfusion, are associated with adverse effects to the recipient. These include the development of febrile transfusion reactions, graft-versus-host disease, alloimmunization to leukocyte antigens, and the immunomodulatory effects that might influence the prognosis of patients with a malignancy. Moreover, it has become evident that such leukocytes may be the vector of infectious agents such as CMV, HTLV-I/II, and EBV as well as other viruses. An interesting development that has occurred coincidentally in transfusion medicine is that agencies responsible for the provision of blood products are being designated manufacturers of biologics. The trend among manufacturers of biologics is to try to produce pure products to provide for the specific therapeutic need of patients. Thus, with the realization that allogeneic leukocytes and their products may have adverse biologic activities, there is increasing pressure from various sources for the reduction of the leukocyte content in allogeneic blood components to minimize the occurrence of their adverse effects. Although the threshold leukocyte number below which these effects might no longer occur is still to be determined, a 2 to 3 log₁₀ leukocyte reduction, provided by the currently available commercial leukocyte filters, has been shown to reduce the frequency of many of such reactions.

On the other hand, the immunosuppressive effects produced by the infusion of allogeneic leukocytes might be beneficial for some patients, ie, for the maintenance of kidney allografts, in possibly reducing the relapse rate in patients with inflammatory bowel diseases, and in ameliorating recurrent spontaneous abortion. Moreover, therapeutic granulocyte transfusions may be of benefit in certain well-defined categories of patients infected with bacteria, yeast, and/or fungi. These include neonates with bacterial sepsis as well as severely neutropenic leukemic patients with an infection unresponsive to appropriate and specific antibiotic therapy.

Many of the results obtained with the use of leukocyte depletion filters are tantalizing, but the actual clinical benefit of leukodepletion has not been established in most instances, because much of the available data are retrospective or from uncontrolled clinical trials. Moreover, issues of cost-effectiveness and quality control have not been considered adequately. Properly designed prospective clinical trials are essential to provide data with which to answer such questions and also to help define the optimal conditions required for the preparation of blood components ultimately destined for clinical use. Only with the availability of such data can sound decisions be made about the clinical value of leukodepletion.

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Biologic effects of leukocytes present in transfused cellular blood products

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