■ CLINICAL CONCEPTS AND COMMENTARY

Richard B. Weiskopf, M.D., Editor

Immunomodulatory Aspects of Transfusion

A Once and Future Risk?

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BLOOD is good for you. What Goethe described as "*ein ganz besonderer Saft*" is the very stuff of life. It is not blood but other people's blood that worries us. We have good reason for this concern. For more than 300 years, the therapeutic promise of blood transfusion has been tempered by the recognition of adverse reactions, often mild, but occasionally acute, dramatic, and lethal. The public hears daily how hepatitis and human immunodeficiency viruses (HIV) continue to spread, despite the best efforts of public health authorities. Unfortunately, blood transfusion played a highly visible, if greatly exaggerated, role in these epidemics. Now an additional transfusion-related threat seems to be emerging. Evidence from a variety of sources suggests that allogeneic blood alters the immune response in a way that may render the recipient vulnerable to infection, the recurrence of malignancy, or the reactivation of latent viruses. This phenomenon has been termed the *immunomodulatory* effect of blood transfusion.

Few current practitioners appreciate that during the first half of the 20th century the major risks of transfusion were immunologic, not infectious.[1] Incredibly, life-threatening hemolytic transfusion reactions, now occur-

ring about once in every 600,000 units transfused, occurred about once in every 500 transfusions earlier in this century. Transfusion safety improved in parallel with our understanding of the humoral immune system. Landsteiner's description of the major blood groups in 1900 removed the dominant immunologic barrier to transfusion and provided the theoretical basis for understanding red cell compatibility. Later, a crude agglutination assay, the antiglobulin (Coombs) tests, furnished the tools for identifying other clinically important antigens expressed on the red-cell membrane. Antiglobulin testing proved critical for detecting alloantibodies, diagnosing hemolytic transfusion reactions, providing serologically compatible blood, and understanding and preventing most cases of hemolytic disease of the newborn. Similar assays defined the platelet and leukocyte antigens that were responsible for post-transfusion febrile reactions and immune refractoriness to platelet transfusion. By the last quarter of the century, most clinicians believed that the immunologic complications of blood transfusion were well understood if not largely solved.

Ironically, as knowledge about the mechanisms of immune responsiveness and tolerance evolves, and as tools to measure alterations in immunity become available, additional immunologic consequences of blood transfusion are being detected. Numerous alterations in circulating blood cells have been reported in patients transfused with allogeneic blood. These changes include decreased numbers of circulating lymphocytes, modifications in the T-cell helper/suppressor ratio, changes in B-cell function, down-regulation of antigen-presenting cells, and activation of immune cells as measured by a number of cell surface markers.[2] Some of these changes persist for months or even longer after transfusion. The lingering question has been whether these observations represent no more than laboratory curiosities, or

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Received from the Department of Transfusion Medicine, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland. Submitted for publication January 4, 1999. Accepted for publication April 28, 1999. Support received solely from institutional and/or departmental sources.

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Key words: Blood; immunology; leukoreduction; tolerance.

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whether they reflect some clinically relevant alteration in the recipient's immune status.

The seminal publication by Opelz *et al.*[3] in 1973 provided clinical evidence that, contrary to the conventional wisdom, blood transfusion prior to renal transplantation improved the survival of cadaver-derived renal allografts. Furthermore, the effect appeared to be dose-dependent; the greater the number of units transfused, the better the chances of graft survival. Experiments in animal models supported this beneficial effect of transfusions in graft outcome. These results have been confirmed in numerous subsequent studies including those of renal grafts from living related donors and cadaveric cardiac transplants. For nearly a decade, deliberate pretreatment with transfusion was practiced at many transplant centers. Some recent reports have suggested that the "transfusion effect" has disappeared in the era of potent immunosuppressive drugs. However, studies of the HLA-DR status of donor and recipient indicate that transfusions matched for at least one of the patient's DR antigens are associated with improved survival of subsequent cardiac or renal allografts. Although few clinicians would advocate deliberate exposure to allogeneic blood for its immunomodulatory effects, a newly completed, large, multiinstitutional, randomized, prospective trial of pretransplant blood transfusion in renal transplantation reports a 9% ($P = 0.025$) graft survival advantage after 5 years in transfused patients.[4] Most investigators now accept the existence of this immunomodulatory effect, although the biologic mechanisms that mediate this effect remain poorly understood.

The role of perioperative blood transfusion in the recurrence of surgically excised tumors and in the survival rates of cancer patients has been disputed for more than a decade.[5] Early retrospective reports of patients with colon cancer, matched for clinical stage, histologic characteristics, and various other factors, indicated that those who received transfusions, particularly those who were heavily transfused, did not fare as well as a nontransfused control group in terms of tumor recurrence, survival, and recurrence-free survival. Studies in both in-bred and out-bred experimental animals indicate that allogeneic blood has tumor growth–promoting activity, and that this activity may be immunologically mediated.[6] More than 60 retrospective clinical studies of transfusion in a variety of tumors have now been published, and slightly more than half of these reports suggests that transfusions affect prognosis adversely. Few suggest that transfusion is beneficial in any way other than to support myelosuppressive therapy. Studies of renal and lung cancer most

often report a transfusion effect. However, investigations of patients with soft-tissue sarcoma, breast, head and neck, or prostate cancer have yielded similar results. Of three prospective controlled trials in patients with colon carcinoma randomized with autologous blood, one showed an adverse effect of allogeneic transfusion and two did not. Finally, four reports have indicated an approximately two-fold increase in non-Hodgkin's lymphoma with blood transfusion, but a large national registry study from Sweden failed to confirm such a link. It is not yet possible to reconcile these findings. The proposed effect may be an artifact of patient selection and study design. A metaanalysis of studies of blood transfusion and colorectal cancer reported a 37% excess risk for transfused patients but concluded that the extent of residual confounding in the data of the 11 observational studies fully explained this difference. However, this reviewer believes that the effect is real, albeit small, and that there may well be some subset of patients, perhaps defined by immune status or tumor subtype, that is particularly susceptible to the effects of allogeneic transfusion. Demonstration of such a difference will likely require a large, carefully controlled, prospective study.

Similar controversy surrounds the relationship between perioperative transfusion and the risk of postoperative infection. Again, animal studies suggest an adverse effect of allogeneic transfusion in experimental surgical models. Almost all of the retrospective studies of transfusion and postoperative infection conclude that transfusion is the single best predictor of postoperative infection. However, the number of confounding variables in these reports complicates interpretation of the results.[8] The results of six prospective, randomized clinical trials are contradictory. Several of these trials suggest that fewer postoperative infections and several-fold reductions in rates of morbidity and mortality occur if leukocytes are removed by filtration from the transfused red cells.[9] A recent randomized clinical trial in the Netherlands reported a significant reduction in postoperative mortality rate in cardiac surgery patients who received three or more transfusions rendered leukocyte-poor by filtration; the effect was only partially explained by decreased numbers of postoperative infections. Whether this finding is real, and whether it is related to transfused leukocytes or to the cytokines produced by leukocytes during storage, awaits additional studies.[10]

The suspicion that transfused leukocytes might somehow be associated with the immunomodulatory effect of transfusion finds support from a variety of sources. First, more than 30 years ago, transfused blood from donors

with positive tuberculin reactivity was shown to transfer reactivity to previously negative transfusion recipients. The transfer of delayed hypersensitivity was attributed to transfused cells or cellular factors. Second, allogeneic leukocytes have been used therapeutically to suppress immune rejection of the fetus by women who have had recurrent spontaneous abortions. Third, immunocompetent transfused leukocytes are known to persist in immunocompromised patients and to cause fatal graft-versus-host disease.[11] Recently, transfused leukocytes have been shown to circulate and undergo an *in vivo* mixed lymphocyte reaction in trauma patients and patients undergoing orthopedic surgery. These patients showed no evidence of toxicity from the circulating mononuclear cells.[12]

Using molecular techniques, Busch *et al.* have observed recently that allogeneic lymphocytes contained in transfused blood have continued to circulate for more than a year in some transfused trauma patients. Similarly, donor lymphocytes have been found to persist for years in recipients of organ allografts, and this "mixed chimerism" has been associated with a relative state of tolerance for patients who have received either bone marrow or solid organ transplants. The observation that mononuclear cells transmitted during pregnancy to a mother from the fetal circulation may circulate for 20 years or more suggests that the tolerance to transfused cells may persist long after the original exposure to allogeneic blood. An interesting association between these cells and the development of systemic sclerosis years after pregnancy has recently been observed.[13] These are provocative findings and provoke speculation that unexpected immunologic effects related to blood transfusion may appear long after the transfusion episode.

The fate of viable lymphocytes in transfused blood may involve one of three courses (fig. 1). Transfused mononuclear cells appear to be sequestered initially in the lung, liver, spleen, and possibly in other reticuloendothelial tissue. Exposure to the recipient's immune cells leads to an "*in vivo* mixed lymphocyte reaction," and although the donor cells may reappear in the circulation and persist for several days, they disappear relatively rapidly, at least as can best be determined by sensitive assays of molecular markers. This scenario is equivalent to graft rejection. In some circumstances, particularly if donor and recipient match closely at major HLA loci, and if the recipient is immunosuppressed, transfused lymphocytes engraft, proliferate, and through both direct cytotoxic activity and cytokine release (interleukin-1 and -2, tumor necrosis factor, interferon, granulocyte-mac-

rophage colony-stimulating factor) attack host cells in a syndrome referred to as *transfusion-associated graft-versus-host disease*. The balance of CD4 lymphocytes differentiate into Th1 inflammatory cells. In the third circumstance, subsets of donor mononuclear cells persist, probably engrafting and proliferating, and circulate in the host for many months and even years. This probably represents a "tolerance" phenomenon. The balance of CD4 lymphocytes probably differentiate into helper Th2 cells. The factors that determine whether an activated CD4 cell will become a Th1 or a Th2 cell are not well defined. The fate of transfused mononuclear cells depends on a variety of factors including histocompatibility, host immune status, dose of infused cells, maturity of host immune surveillance, and possibly the presence of microbial pathogens. Exactly how the immune balance is tilted toward one or another of these pathways is not clear. The role of these "passenger lymphocytes" in the immunomodulatory effects of blood transfusion is an area of intense investigational interest.

The cause of the immunomodulatory effects of blood transfusion remains undetermined. If allogeneic leukocytes, whether lymphocytes, monocytes, or dendritic cells, do play some role, the imminent introduction of universal leukocyte reduction of cellular blood components may decrease or eliminate this risk. However, there are numerous other candidate causes of transfusion-associated immunomodulation. Allogeneic transfusion is associated with the development of networks of antiidiotypic antibodies and of suppressor cells in the recipient. Cytokines of several types accumulate in cellular blood components during storage. Plasticizers from blood containers leach into stored components, and at least one of these, di(2-ethylhexyl)phthalate, has been implicated in development of tumors in rodents; however, the concentrations of plasticizer in blood are several orders of magnitude lower. Allogeneic blood clearly contains viruses of unknown significance, for example the so-called hepatitis G virus and TT virus, as well as agents not yet identified. Some of these may turn out to have immunosuppressive effects. Allogeneic stimulation from transfusion may reactivate a variety of latent infectious agents, including HIV, Epstein–Barr virus, and cytomegalovirus, in the recipient.[14] Clinical studies suggest that heavily transfused AIDS patients suffer increased numbers of infections and rapidly progressive disease. Reactivation of other agents, some with immunosuppressive or oncogenic activity, is certainly conceivable. Finally, high concentrations of plasma protein in crude factor VIII preparations have been implicated in the
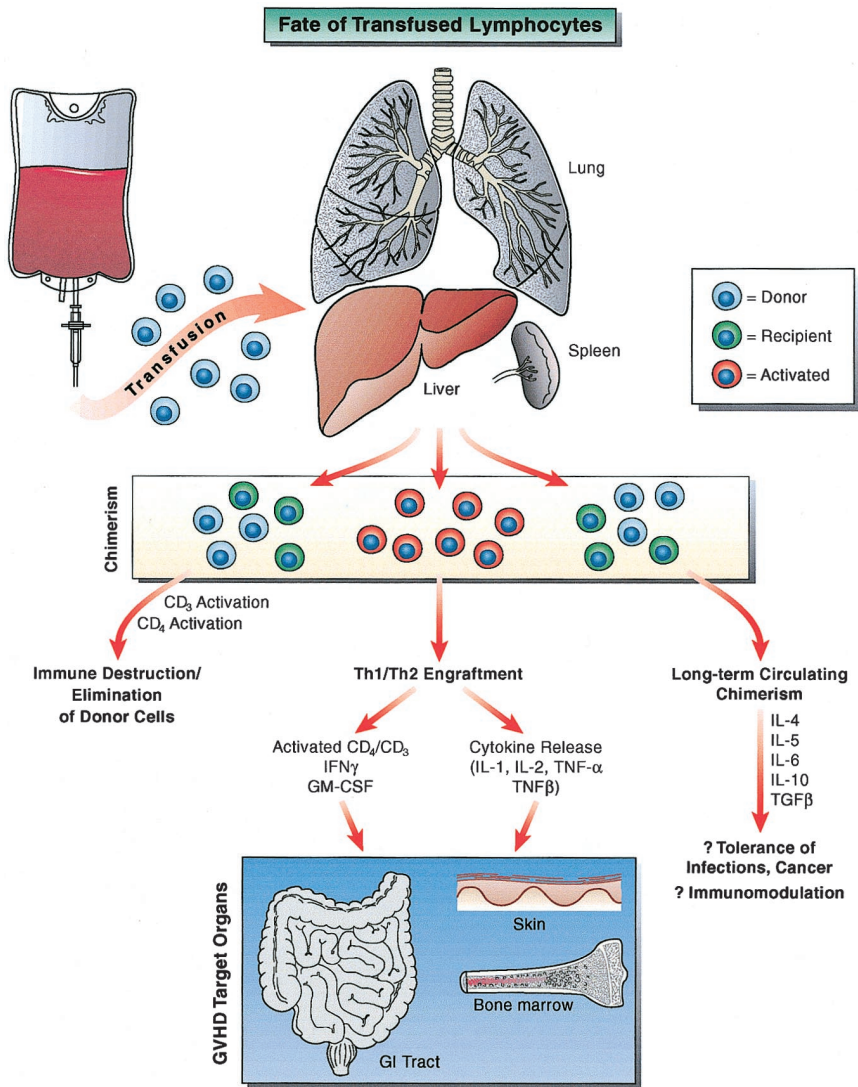
Fig. 1. Fate of transfused lymphocytes. Th = T helper; IFNγ = interferon-γ; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; TNF = tumor necrosis factor; TGFβ = transforming growth factor-β.

immune suppression of some patients with hemophilia A. Any one or a combination of these factors might be involved in immunomodulation.

Based on the sum of evidence, immunomodulation seems likely to be added to the list of unintended effects of allogeneic blood transfusion. The magnitude and importance of these effects, the causative agents, and the patients or patient groups that are at particular risk have yet to be defined. Certainly the risks do not now seem to rival even the much reduced risks of immune hemolysis, acute lung injury, and transfusion-transmitted infection. However, the long-term consequences may be significant. Immunomodulation is yet another reason to use blood transfusion judiciously, but not an excuse to avoid transfusion if it is necessary. Experience with Jehovah's Witnesses, who decline transfusions for religious reasons, has shown that the mortality rate is increased for patients with severe anemia who refuse the blood that physicians consider necessary.[15] Until safer alternatives to allogeneic blood are developed, the decision to transfuse will continue to depend upon that risk–benefit calculus known as clinical judgment.

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