Wolf in Wolf's Clothing: Is it Time to Raise the Bounty on the Passenger Leukocyte?

By Harvey G. Klein

“Most of us have a tendency to view transfusion as a ready means of correcting a deficiency in red blood cells, and so regard the material, administered intravenously, merely as a suspension of red cells, habitually disregarding the other ingredients, or at least minimizing their importance. We rarely consider the fact that the patient receives plasma, leukocytes and more or less damaged platelets in addition to the needed red cells.”

Peripheral blood leukocytes were not designed for blood transfusion. Leukocytes have evolved into the roving marauders, predators, and scavengers of the circulation; the very characteristics and functions that define these specialized cells have made them the most difficult blood cells to domesticate. Not only are leukocytes troublesome to harvest, purify, and store, but once transfused, they may turn upon their host and unleash endogenous pyrogens, cell-associated viruses, or even lethal graft-versus-host disease (GVHD). Indeed, toxicity, rather than lack of efficacy, is probably responsible for the much diminished clinical role of white blood cell (WBC) transfusions.

Nevertheless, current transfusion practice exposes patients to large numbers of allogeneic WBCs. Residual leukocytes are concealed in most blood components and transfused without therapeutic intent. The average unit of red blood cells (RBCs) contains approximately $10^8$ leukocytes, each random donor platelet unit about $10^7$ leukocytes, and some plateletpheresis concentrates $10^6$ leukocytes or more. Until recently, these passenger cells attracted little attention. How times have changed. New data, new technology, and new-found cautions have all focused attention on passenger leukocytes and the rationale for removing them.

Clinicians have long appreciated that WBCs in transfused blood can cause fever and rigors. Transfusions and pregnancy may stimulate alloantibodies directed against a variety of human leukocyte antigens. While immunemediated febrile reactions are disconcerting to patients and their physicians, most are self-limited, rarely dangerous, and usually remedied by reducing the leukocyte content to less than $5 \times 10^9$ cells, a level achieved easily with a variety of techniques. Relatively few patients have repeated febrile transfusion reactions, but those who do, e.g., transfusion-dependent thalassemia patients, can be managed with leukocyte-reduced components. Patients who continue to react to platelet infusions generally require selected donors, because febrile reactions and poor clinical response often go hand-in-hand.

Alloimmunization by passenger leukocytes renders some patients unresponsive to platelet transfusions. Persuasive experimental evidence supports the notion that reducing the incidental lymphocyte content of cellular infusions to less than $5 \times 10^9$ cells will markedly reduce or eliminate primary sensitization. Recently developed cell separation techniques and specially designed filters can achieve this goal. Because the new technology is costly and the predicted benefits possibly small, firm recommendations concerning prophylactic leukocyte reduction to prevent platelet refractoriness seem premature. The outcome of a large multicenter controlled clinical trial (the TRAP study) should help clarify some of these issues.

There is a burgeoning if somewhat opaque body of literature concerning the “immunomodulatory” effects of allogeneic blood transfusion. Numerous reports catalogue changes in the recipient’s immune response, including cellular and humoral markers of lymphocyte activation. Circumstantial evidence implicates passenger leukocytes in some of these changes. Whether such alterations in any way influence host responses to malignancy, infection, or autoimmune disease, and whether modification of blood components influences these changes, remains highly speculative.

Perhaps the most feared and, fortunately, rare immune complication involving immunocompetent passenger lymphocytes is transfusion-associated GVHD. As few as $10^6$ lymphocytes may result in fatal GVHD in a susceptible recipient. Although leukocyte depletion probably decreases the risk, transfusion-associated GVHD has been reported in at least one patient who received only leukocyte-reduced components. Reliable prophylaxis for GVHD still requires gamma irradiation of blood components.

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Increasingly, leukocytes are regarded as important vectors of transfusion-transmitted cell-associated viruses. There is less consensus about the risk such infections pose to the average transfused patient and the potential benefit of reducing the number of leukocytes in cellular blood components. The most frequently recognized pathogen appears to be cytomegalovirus (CMV), a herpesvirus generally transmitted in a latent, noninfectious state and reactivated after transfusion. Because CMV seroprevalence rates in the United States vary from 30% to 80% and because most seronegative donors do not appear to be infectious, transfusion infrequently results in primary CMV infections. Furthermore, clinical problems from primary infection occur principally in selected patient groups such as seronegative pregnant women, transplant candidates, acquired immunodeficiency syndrome (AIDS) patients, and neonates; these patients usually receive CMV-seronegative blood components. Although the minimum number of cells capable of transmitting infection is unknown and may depend on both donor and recipient factors, filters that reduce the leukocyte content by three logs (99.9%) may diminish or prevent the risk. Leukocyte reduction might also decrease transfusion-related primary infections with other cell-associated viruses such as Epstein-Barr virus (EBV) and the human T-cell leukemia viruses I and II (HTLV I/II), although the former is not often implicated in transfusion-associated disease and infection with the latter has been rare since the introduction of fairly sensitive and specific donor screening tests. Finally, laboratory evidence suggests that leukocyte reduction might reduce the infectivity of human immunodeficiency virus type 1 (HIV-1)-seropositive units, but clinical confirmation that filtered blood components decrease HIV-1 infections has not been forthcoming. Overall, leukocyte reduction might prevent some transfusion-transmitted infections, but the practical benefit is difficult to predict.

In this issue of Blood, Busch et al present provocative studies suggesting that the residual WBCs in blood components may have other important adverse effects. In their report, donor leukocytes cocultured with lymphocytes from HIV-seropositive patients induce HIV-1 replication in a dose-dependent fashion. Partially purified allogeneic RBCs, platelets, and plasma from the same donors do not. Furthermore, viral reactivation is followed by dissemination of HIV-1 to previously uninfected patient cells. Companion experiments show that contact with allogeneic donor peripheral blood mononuclear cells upregulates HIV-1 expression in a latently infected cell line and increases the susceptibility of heterologous blood mononuclear cells to exogenous HIV-1 infection. These data complement earlier reports that a variety of stimuli activate infected T cells, upregulate HIV-1 replication, and result in in vitro viral dissemination.

How might these findings affect clinical transfusion practice? One reasonable hypothesis is that leukocyte-containing blood components transfused to a recipient with HIV-1 infection activate latent HIV-1 in a dose-dependent fashion and accelerate HIV-1 spread and disease progression. Reports that, of patients infected with HIV-1 by blood transfusion, those most heavily transfused progress most rapidly to AIDS, and that persons infected through transfusion develop AIDS sooner than do those infected through other routes, are consistent with this interpretation. The hypothesis can be examined in part by applying quantitative assays of viral activation and spread to HIV-1-positive patients transfused with either unmodified or leukocyte-reduced blood components, as Busch et al have recommended. Confirmation requires at the very least a carefully crafted clinical trial with quantitative laboratory endpoints. If these findings are confirmed in vivo, leukocyte-reduced blood components should be considered for HIV-1-infected patients who require transfusion. Definitive randomized, controlled clinical studies with disease progression endpoints, while desirable, would be difficult to justify. While conclusive evidence that viral load predicts disease progression is lacking, increasing viral burden in peripheral blood CD4+ T cells has been directly associated with a progressive decline in CD4+ lymphocytes and with a deteriorating clinical course in HIV-infected patients.

The report of Busch et al raises other important questions. What is the mechanism of activation and does it have implications for other latent viral infections? The link between cellular activation and HIV regulation is poorly understood. While viral latency has evolved as a mechanism by which viruses evade detection by the host, it is likewise to the host’s advantage to maintain the virus in a quiescent state. HIV-1 and a number of other blood-borne viral pathogens (CMV, EBV, and hepatitis B virus) express their genome only sporadically, even when integrated into host DNA. Some asymptomatic individuals appear to harbor HIV-1 as unintegrated DNA in a reservoir of quiescent T lymphocytes. Reactivation of HIV-infected peripheral blood lymphocytes by mitogens, antigens, and selected cytokines has been shown to induce re-expression of the virus. Other transfusion-transmitted lymphotropic viruses may also play a role in HIV reactivation, in HIV spread, and even in the selection of HIV strains of increased virulence. Transfusion-related lymphocyte stimulation likely affects other latent viral infections as well. For example, allogeneic but not syngeneic blood and leukocyte transfusions have been associated with the reactivation of CMV in a murine model. Human T lymphocytes infected with CMV in vitro produce virus when cocultured with allogeneic lymphocytes, but not when cocultured with autologous cells. Whether allogeneic transfusion might have a clinically significant effect on other unrecognized silent infections is still a matter of conjecture.

If allogeneic leukocytes accelerate clinical infection, the implications for HIV-1 infection alone are dismaying. More than 230,000 patients with AIDS have been reported in the United States and they will receive an estimated 200,000 units of RBCs cells in 1992. Autologous blood is usually not an option, and recombinant human erythropoietin therapy is effective in only a fraction of these patients. An estimated 1 million Americans are infected with HIV-1 and most will be unaware of their infection should they require
transfusion for some intercurrent illness. If treatment of blood components will decrease the reactivation in asymptomatic HIV-1 carriers and slow disease progression in patients with advanced infection, serious thought should be given to removing leukocytes from allogeneic cellular components. Any benefit regarding alloimmunization, primary infections, GVHD, and activation of other latent viruses would be a bonus.

Strategies for improving the safety of allogeneic blood transfusions have traditionally emphasized the conservative use of blood, donor screening, laboratory testing, and sterilization of blood components. Each of these approaches has enjoyed a measure of success. Blood quality also relies upon the removal of recognized impurities. Even as evidence mounts that passenger leukocytes may be detrimental to an increasing number of recipients, practical technology for removing these “cellular impurities” has emerged. Commercially available blood filters can extract two to three logs of residual leukocytes from blood components with a small loss, and no obvious damage to the RBCs or platelets. Filters that remove four to six logs are in development. For medical science, the mechanism of latent virus activation is vitally important, but for practical purposes, perhaps it is enough to show that leukocytes can cause it and removal of leukocytes can prevent it. We have the ability to remove them now. That may be enough to keep the wolf from the door.

REFERENCES

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