Prevention of Alloimmunization Against Platelets

ALLOIMMUNIZATION represents the main clinical problem limiting the benefits of platelet transfusions to patients with leukemia and other bone marrow failure states. Alloimmunization is characterized clinically by the failure to obtain adequate platelet count increments and should be suspected if platelet counts 10 to 60 minutes after transfusion are compromised. The clinical diagnosis can be confirmed serologically by the measurement of antibodies against class-I HLA antigens, usually using a standard lymphocytotoxicity assay. Antibody against platelet-specific antigens is the cause of refractoriness in only a small fraction of patients, although antibody techniques using the platelet as the target cell can also be used to confirm the presence of clinically relevant alloantibody.

Studies of patients with acute myeloid leukemia (AML), conducted at our institution in the 1970s, showed alloimmunization in approximately 40% to 50% of newly diagnosed patients receiving multiple random donor platelet transfusions. More recently, only about 25% to 30% of patients with AML have become refractory to random donor platelet transfusions. Antibody levels and specificity can also decrease with time, so that some patients can be transfused successfully with random donor platelets once again. The development of alloimmunization is independent of the number of transfusions administered; patients also tend to become alloimmunized early in the treatment course. If alloimmunization has not developed within the first 4 to 6 weeks of chemotherapy and transfusion, antibody infrequently develops later despite repeated transfusions. Thus, it is possible at the end of induction therapy, when decisions about further chemotherapy treatment programs are being made, to predict difficulties in transfusion support in individual patients.

The management of alloimmunized patients is problematic. The standard approach is to administer closely HLA-matched platelets obtained by single donors using apheresis technology. Despite refinements of methods of donor selection, as many as 40% to 60% of “histocompatible” transfusions administered to alloimmunized patients are unsuccessful. In addition, it is often difficult to identify large numbers of HLA-typed donors for individual patients, particularly for patients with unusual HLA types. There is a suggestion that cross-matching using platelet antibody testing can supplement and improve on these results, but few large, prospective trials have been performed. There are no proven approaches to the management of bleeding in alloimmunized patients when histocompatible donors cannot be located. Therapeutic maneuvers that are successful in immune thrombocytopenic purpura, such as the administration of high-dose intravenous gamma globulin, are ineffective in alloimmunized patients.

Thus, any means by which alloimmunization could be eliminated would be of considerable clinical consequence, particularly because chemotherapeutic approaches in patients with AML and acute lymphocytic leukemia have more recently focused on intensive postremission therapy, including bone marrow transplantation. Perhaps what is most biologically interesting is that the majority of patients receiving large amounts of antigen do not form antibody and behave as if they were “immune tolerant.” There is a large body of in vitro and preclinical evidence suggesting that the leukocytes contaminating platelet suspensions are the primary stimulus for alloimmunization. Dausset and Rapaport demonstrated that highly purified platelet preparations injected intradermally failed to induce sensitization to skin grafts from the same human donors, whereas lymphocytes from the same donor reliably induced sensitization with rejection of subsequent skin grafts. Analogous results were observed in murine models, with the additional observation that the injection of purified platelet preparations alone produced a state of tolerance. Although the mechanism is poorly understood, it appears that presentation of class I and class II antigens by intact, viable leukocytes is required for initial processing by the immune system; platelets do not express class-II (Dr) histocompatibility antigens. More recently, it has been shown that UV-B irradiation can also abrogate the ability of lymphocytes to serve as stimulating cells both in vitro and in vivo. Doses of UV-B irradiation sufficient to abolish reactivity in mixed lymphocyte reactions do not affect platelet function in vitro or the posttransfusion survival of radio-labeled autologous platelets. Canine recipients of UV-B irradiated platelets had a much lower incidence of alloimmunization compared with control animals.

Based on these compelling preclinical observations, there has been considerable interest in the clinical application of different methods of leukocyte removal or modification to reduce the incidence of alloimmunization. In an early controlled study performed at our institution, there was no significant reduction in alloimmunization when leukocyte depletion was performed by simple centrifugation techniques. However, it is likely that centrifugation did not remove sufficient numbers of contaminating leukocytes from the platelet concentrates (PC). Other, uncontrolled
observations in The Netherlands by Eernisse and Brand demonstrated a low rate of alloimmunization when less than 10\(^7\) leukocytes were administered per transfusion.\(^{33,34}\) A cumbersome method of leukocyte depletion was used that is not readily usable in most blood bank settings. More recently, a number of platelet filters have been developed that seem to be capable of reducing leukocyte contamination to similar levels\(^{35,36}\); the report by van Marwijk Kooy et al.\(^{1}\) in this issue of Blood is one of a number of recent publications using these platelet filters.\(^{3,8,12}\)

These are difficult clinical trials to perform, particularly because of the serious intercurrent problems these patients develop. An “ideal” trial should include: prospective randomization with a control group receiving standard, pooled platelet concentrate transfusions; newly diagnosed patients with the same underlying disease, receiving identical chemotherapy; stratification for known predisposing factors, including prior pregnancies or transfusions; objective criteria for alloimmunization, including the development of clinical refractoriness as well as serologic end points that are monitored on a weekly interval; exclusion of alloimmunized patients from randomization or evaluation; standard approach to red blood cell (RBC) transfusions, which includes administration of leukocyte-depleted RBCs; planned registration of adequate numbers of patients to show either significant differences or equivalence; appropriate quality control of the platelet product with selection of a therapeutic maneuver that would be applicable to blood banks of different sizes and sophistication.

van Marwijk Kooy et al report a well-conducted and carefully analyzed study of a total of 53 patients randomized to receive filtered RBCs and either filtered platelets (<5 \(\times\) 10\(^7\) leukocytes/transfusion) or platelet concentrates that had been moderately leukocyte depleted by centrifugation. There was a significant reduction in both clinical refractoriness and anti-HLA antibody formation in the patients receiving leukocyte-depleted platelets. Similar conclusions have been suggested in three other prospectively randomized trials\(^{8,38}\) and in another report using historical controls.\(^{39}\) However, it is not clear that these conclusions can be extrapolated to the leukemia population at large. All of the published trials were small, with 26 to 69 total evaluable patients per study. Two investigators used single-donor platelets either exclusively,\(^{17}\) or in some patients,\(^{2}\) and different methods with variable efficiency and quality control were used to provide leukocyte-depleted RBCs. In addition, in every report the patient diagnoses, and presumably the treatments, were very heterogeneous. For example, approximately 30% of the patients in the current study had acute lymphocytic leukemia and it has been noted that the alloimmunization rate in such patients is lower, probably because of the administration of high doses of corticosteroids during induction therapy.\(^{6,12}\) Perhaps most critically, patients who had received recent transfusions and females with a prior history of pregnancy were excluded from randomization. In the United States, the diagnosis of acute leukemia is frequently made at outlying hospitals from which patients are transferred to referral centers after transfusions are administered. In addition, the median age of patients entered on recent AML studies is 50 to 55 years; approximately half of the patients are female, a high fraction of whom have had prior pregnancies. Therefore, it is unknown whether the same results could be achieved in this large, potentially presensitized patient population.

There are also inconsistencies in the results obtained with comparable amounts of leukocyte contamination. Andreu et al reported only a 12% rate of alloimmunization using PC with the relatively high mean leukocyte “dose” of 36 \(\times\) 10\(^7\)/transfusion after filtration.\(^{5}\) Similarly, Murphy et al\(^{38}\) used an inefficient centrifugation method of leukocyte depletion resulting in products with means of 0.9 to 2 \(\times\) 10\(^7\) leukocytes/transfusion, but with a 16% alloimmunization rate. However, a similar leukocyte count in the control group of the present study resulted in a 42% incidence of HLA alloimmunization. This discrepancy also attests to the variable effectiveness of different methods of PC filtration and possibly to the difficulties in accurately doing leukocyte counts at these very low concentrations.\(^{33}\) Newer filters seem to reliably produce pooled PC transfusions with less than 10\(^6\) leukocytes and may help address some of these technique-related issues.\(^{39}\) There is, however, an approximately 15% to 20% loss of platelets after filtration, potentially increasing the cost because of the larger number of PC that may have to be processed to produce transfusions with sufficient numbers of platelets.

It is also important to consider what fraction of patients might benefit from any approach to reduce the rate of alloimmunization. Of 100 newly diagnosed patients with AML, approximately 10% will be alloimmunized on admission or after their first or second transfusion because of an anamnestic antibody response. This would leave a total of approximately 90 patients who could benefit from any approach to modify alloimmunization. If the baseline alloimmunization rate is 40%, the number of patients who might benefit is reduced to 36. The clinical effect of reduction of alloimmunization is actually most important during postremission therapy. Not all patients are candidates for aggressive subsequent therapy. If one assumes a complete response rate of 75% with 75% of complete responders going on to intensive consolidation therapy, the number of patients is reduced to approximately 21. A fraction of patients receive therapeutic granulocyte transfusions that are not routinely HLA matched, further decreasing the number of patients to 18 or 19. Lastly, it is unlikely that any approach to modify alloimmunization would be 100% effective. If one assumes a two-thirds reduction of the alloimmunization rate, one is left with approximately a dozen patients who might be helped by any such approach. Currently, it is impossible to distinguish prospectively between patients who are more or less likely to become alloimmunized, and it will therefore be necessary to “treat” all patients to benefit a maximum of only 10 to 15 individuals.

Given these considerations, it would be appropriate to require that any new approaches to decrease alloimmuniza-
tion be relatively inexpensive, technically reproducible, and simple to administer. Filters to remove leukocytes from platelets are quite expensive and any such program also entails the use of leukocyte-depleted RBCs, further increasing the costs and administration burdens on blood banks. Because of these issues, UV irradiation has a particular appeal because the technique is relatively simple (potentially involving the push of a button), and could be widely applied with little additional training or personnel costs. A large, randomized multi-institutional study (Trial to Reduce Alloimmunization to Platelets [TRAP]) supported by the National Heart, Lung and Blood Institute has been begun in the United States, testing both leukocyte depletion by filtration and UV irradiation in a group of adult patients, with AML, undergoing initial induction therapy. Until these or other data are available, it is premature to recommend platelet filtration for routine use at this time.

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