Suppression of Transfusion-Related Alloimmunization in Intensively Treated Cancer Patients

By Thomas V. Holohan, Paul I. Terasaki, and Albert B. Deisseroth

A retrospective review of HLA antibody testing and transfusion records of 100 cancer patients who required extensive platelet support revealed that 27 of 100 patients exhibited positive HLA antibody tests; only 13 remained positive on repetitive examination, while 88% of aplastic anemia patients so tested were positive. Sixty-five patients with leukemia, 16 with Ewing’s sarcoma, and 19 with recurrent undifferentiated lymphoma were studied. Each patient received at least 10 U of platelets (mean of 88 ± 7/8 after a mean of 44 U of platelets were transfused. Granulocyte transfusions given therapeutically for granulocytopenia and documented infection did not appear to influence HLA antibody formation. These data indicate that significant immunosuppression occurs in intensively treated cancer patients, as measured by their ability to form antibodies to HLA antigens expressed on the surface of transfused platelets.

Chemotherapeutic agents used in cancer treatment have been shown to cause at least transient diminution of primary and secondary antibody response, cell-mediated immune response, and delayed hypersensitivity; however, recovery from chemical immunosuppression is fairly rapid, especially when intermittent intensive combination chemotherapy is employed. Immunosuppression of varying degree has also been observed in patients with malignancies. In most cases, this is progressive in cancer patients affected with advancing solid tumors and hematologic malignancies.

Of particular significance in this regard is the current supportive management of intensively treated cancer patients in whom thrombocytopenia becomes a clinically significant problem. Although alloimmunization does not invariably accompany frequent red cell or whole blood transfusions in nonmalignant diseases, it has been well established that repeated platelet transfusions to patients with primary bone marrow failure will result in the formation of antibodies to HLA antigens found on the surface of platelets. There are few similar studies in patients undergoing chemotherapy for malignant disease. Consequently, there are only sparse data addressing the question as to whether disease-related and chemical immunosuppression restricts alloimmunization, or whether antibody formation is directly a function of transfusion history. Most of such data as exists relates to patients with acute leukemia. Some investigators have described virtually complete impairment of transfusion-related antibody formation, while most have reported fairly high alloimmunization frequencies, ranging from 50%–100% of all patients studied. However, for the most part, these studies encompassed fairly small samples, and follow-up data were generally not available for the entire duration of transfusion support. This latter factor may be of significance, since there is some indication that impairment of immune response or immunologic tolerance may increase over the course of treatment.

In the present study, we retrospectively reviewed the transfusion records and HLA antibody screening of 100 patients with malignant disease.

Materials and Methods

One-hundred patients with malignant disease treated at the Pediatric Oncology Branch of the National Cancer Institute (NCI) were chosen for this study. Patients were selected on the basis of relatively frequent requirements for platelet transfusion secondary to intensive chemotherapy. Included were patients treated for acute lymphocytic leukemia with various protocols since 1968, all patients with recurrent/relapsed undifferentiated lymphoma treated with “BACT’ therapy (BCNU, cytosine arabinoside, cyclophosphamide, and 6-thioguanine), and all patients treated with our current protocol for “high risk” (metastatic or central axis) Ewing’s sarcoma. The latter group received intensive combined modality therapy, including radiation therapy to the primary and metastatic lesions, total body irradiation (150 rad), and chemotherapy including the drugs vincristine, actinomycin D, cyclophosphamide, adriamycin, and DTIC. Marrow reconstitution using cryopreserved autologous bone marrow was provided. The mean ages for patients in these groups were 14 yr for acute leukemia, 17 yr for lymphoma, and 20 yr for those with Ewing’s sarcoma. The mean body surface area for all 100 patients was 1.53 sq m.

Random (non-HLA matched) single donor collections were utilized for transfusion. “One unit” was defined as the quantity of platelets extracted from the platelet-rich plasma of 1 U of donor whole blood. During each donation procedure, four such units were

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cases administered as 4-U random single donor transfusions. Eligibility for inclusion in this evaluation required the following: (1) no HLA antibodies detectable at entry; (2) transfusion of a minimum of 10 U of platelets; (3) repeated HLA antibody testing during therapy, each of which had to be drawn within 6 wk of administration of platelets. Any platelets given after the last antibody detection test for a given patient were not included in the analysis. HLA antibody detection tests were performed by lymphocytotoxicity method against a panel of 83–100 cells by one of the authors (P.I.T.). A positive result was considered to be reactivity against at least 10% of the panel cells (in fact, the majority of positive tests were cytotoxic to more than 40% of the panel). By the above criteria, there remained 65 evaluable patients with acute leukemia, 19 with lymphoma, and 16 with Ewing’s sarcoma. In addition, a concurrent analysis of a small group of aplastic anemia patients followed by NCI and meeting the same criteria was performed. The Kruskal-Wallace one-way analysis of variance and point-biserial correlation coefficient techniques were employed for statistical analysis of the data.

RESULTS

Our analysis has shown that in contrast to aplastic anemia patients in whom 88% (7/8) were persistently positive for HLA antibodies, only 13% of our cancer patients exhibited stable antibody positivity, suggesting that this type of patient may be less responsive to platelet alloantigens to which they are exposed during platelet support.

Acute Leukemia

Sixty-five leukemia patients were followed for a mean of 415 days (range 16–2199) and received a mean of 139 U of platelets. A mean of five antibody detection tests were performed for each patient. Forty-five patients, or 69% of the total group, never developed a positive reaction on this testing (Table 1).

This subgroup of antibody-negative patients received an average of 74 U of platelets (median 31) over a period of 214 days, with 4 negative antibody tests per patient performed during that period. Ten patients developed anti-HLA antibodies, but did not remain alloimmunized during the remainder of their course of treatment. Antibodies were detected after a mean of 41 U of platelets (median 31) were given over 105 days. They were present for only 59 days, at which time reversion to an antibody-negative status occurred. These patients did not again evidence antibodies during the following (mean) 303 days of observation, during which they required an average of 146 U of platelets each. The remaining 10 patients developed HLA antibodies and remained alloimmunized during their entire period of follow-up. Antibodies in this group were detected after a mean of 119 U of platelets were given (median 83, range 28–321) over a median interval of 499 days.

Twenty-four patients in the acute leukemia group received granulocyte transfusions during their course of therapy. Thirteen of these belonged to the antibody-negative group; of the remaining 11, 7 were antibody negative before, during, and at least 6 wk after transfusion of white cells, two were antibody positive prior to administration of granulocytes, and two developed antibodies in temporal association with white cell transfusions. The mean number of granulocyte transfusions administered was 10.5 (median 7); the mean number given to the antibody-negative group was 9.7. Of the two patients converting to antibody positivity in association with granulocytes, one received 20 transfusions, the second, 1.

In an attempt to determine if the differential antibody formation was related to the number of platelets transfused, comparisons were made between the total number of platelet units given to the antibody-negative group, and the number of platelet units administered to the other two groups up to the point at which HLA antibodies were first detected. A (corrected) Kruskal-Wallace one-way analysis of variance was performed; a chi-square value of 4.00 was obtained, corresponding to a “p” value of 0.15 (for df = 2). A point-biserial correlation coefficient, relating antibody status to platelets transfused, was calculated as 0.03. One cannot, therefore, conclude that the antibody negative group received significantly fewer platelets, nor that there seemed to be any discernible relationship between alloimmunization and magnitude of platelet support.

In reviewing the records of all leukemia patients, six cases were found where HLA antibodies were formed without prior exposure to platelet or white cell transfusions. All six were antibody negative at entry, and became antibody positive after transfusion of packed red cells. The mean number of units of red cells transfused prior to the change in antibody status was 5.5 (median 2.5). These patients were unevaluable by the criteria for this study, as antibody positivity was present prior to platelet therapy.

Ewing’s Sarcoma

The current NCI protocol for high-risk Ewing’s sarcoma was established in 1977, and 16 patients were
until antibodies detected.

of platelets transfused was significant at the 0.001 way analysis of variance. The difference in the amount of platelet transfusion between antibody positive and negative groups was made by a Kruskal-Wallace one-

5 received a mean of 67 U of platelets (median 29 days (median 26) prior to antibody detection of pl4elets over 41 days.

This individual formed antibodies after transfusion of 2 U of packed red cells; he later became antibody-negative despite transfusion of 88 U of platelets over 41 days.

Recurrent/Relapsed Lymphoma

Twenty-three patients were treated for recurrent or relapsed lymphoma on the BACT protocol; of that number, 19 were evaluable. These patients received a mean of 83 U of platelets (median 51) over a mean interval of 78 days (median 24), during which time an average of 4 antibody detection tests were performed per patient. Five developed HL antibodies; these alloimmunized patients had been transfused a mean of 36 U of platelets (median 40) over an average interval of 29 days (median 26) prior to antibody detection (Table 3). The patients remaining antibody negative received a mean of 67 U of platelets (median 51) over a mean interval of 67 days (median 22). Comparison of platelet transfusion between antibody positive and negative groups was made by a Kruskal-Wallace one-way analysis of variance. The difference in the amount of platelets transfused was significant at the 0.001 level, indicating significantly less transfusion support in the alloimmunized group. Likewise, a point-biserial correlation coefficient between antibody status and amount of platelets transfused was 0.45, revealing that higher levels of platelet support were associated with the absence of formation of HL antibodies. These statistical analyses indicated that antibody negativity could not be attributed to relatively less transfusion exposure. Of the five alloimmunized patients, three reverted to an antibody negative status and remained so despite further transfusion of a mean of 81 U of platelets over an average interval of 27 days.

Three patients were given granulocyte transfusions during the period of platelet support (mean of 17 transfusions each); all granulocyte recipients belonged to the group of patients that remained antibody negative. As in the acute leukemia group, there appeared to be no increased risk of alloimmunization with the administration of white cells.

Considering all 100 patients across subgroups then, 27 (27%) developed HL antibodies; of that number, 14 reverted to an antibody negative status in the face of continued transfusion. When all patients who had antibody detection tests performed less than 2 wk after the initiation of platelet transfusion were eliminated from consideration so as to minimize the number of “false negative” cases, it was found that 25 of 94 (27%) recipients developed alloantibodies (19/63 leukemias, 2/15 Ewing’s patients, and 4/16 lymphoma patients), and of those 25, 13 reverted to an antibody negative status. Therefore, only 13% of all patients evaluated developed HL antibodies and maintained that status while transfusion therapy was continued.

The mean number of units of platelets given to all antibody negative patients was 71 (median 50); the alloimmunized patients received a mean of 76 U (median 40) to the point at which antibodies were first detected. A Kruskal-Wallace one-way analysis of variance was performed and revealed no significant difference in the quantity of platelets transfused between the antibody positive and negative groups (chi-square 0.213). A point-biserial correlation coefficient between antibody status and magnitude of platelet administration was calculated as 0.03. Therefore, no

### Table 2. Platelet Support, Ewing’s Sarcoma

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Units of Platelets (Mean)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Ewing’s sarcoma</td>
<td>16</td>
<td>82 (median 67)</td>
</tr>
<tr>
<td>Antibody negative</td>
<td>14</td>
<td>67 (median 53)</td>
</tr>
<tr>
<td>Antibody positive</td>
<td>2</td>
<td>138 (256, 20) (median 123)</td>
</tr>
</tbody>
</table>

*For antibody positive represents number of units of platelets given until antibodies detected.

### Table 3. Platelet Support, Undifferentiated Lymphoma

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Units of Platelets (Mean)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All lymphoma</td>
<td>19</td>
<td>83 (median 51)</td>
</tr>
<tr>
<td>Antibody negative</td>
<td>14</td>
<td>67 (median 51)</td>
</tr>
<tr>
<td>All antibody positive</td>
<td>5</td>
<td>36 (median 40)</td>
</tr>
<tr>
<td>Reversion to negative</td>
<td>3</td>
<td>37 (median 40)</td>
</tr>
<tr>
<td>Remaining positive</td>
<td>2</td>
<td>34 (median 34)</td>
</tr>
</tbody>
</table>

*For antibody positive represents number of units of platelets given until antibodies detected.
significant relationship could be established between antigen (platelet) exposure and HLA antibody status.

Among our patients who became alloimmunized, the shortest interval from initiation of repeated platelet transfusion to the detection of antibodies was 11 days, and the longest well over 1 yr. The minimum number of units transfused prior to antibody positivity was 10, and the maximum 321.

We also examined the relationship of alloimmunization to both sex and the HLA-A2 antigen. Our sample was comprised of 65 males and 35 females. HLA antibodies were detected in 22% of males and 37% of females (see Table 4). This sex-related difference was not statistically significant. The presence of the HLA-A2 antigen was unrelated to alloimmunization. Antibody positivity was noted in 20% of patients possessing, and 25% of those lacking, this antigen. The frequency of anti-HLA antibodies was calculated for those 13 patients who exhibited persistent alloimmunization (Table 5). Although the sample size is small, it would appear that the likelihood of alloimmunization to a particular HLA antigen is related to the frequency of occurrence of that antigen in the general population.

After completing the above analysis on our cancer patients, we reexamined the relatively small number of patients with aplastic anemia who have been followed at the POB, NCI. Eight patients were found who met the criteria established for evaluation of the patients with malignancies reported here. These aplastic patients received comparable levels of platelet support during periods when such transfusions were clinically indicated, and 7 of the 8 (88%) developed HLA antibodies. Alloimmunization occurred after transfusion of a mean of 44 U of platelets (median 30) over an average interval of 44 days. These data are in accord with the historical information regarding alloimmunization in this disease.11-15,24,25 In terms of the quantity of platelet support for the alloimmunized patients (to the point at which HLA antibodies were first detected) and the antibody negative patients (total platelets transfused), chi-square tests between the aplastic patients and both the antibody positive and negative cancer patients revealed no significant differences. Point-biserial correlation between platelet support and antibody status was 0.17 for aplastic versus antibody negative cancer patients. Therefore, our patients were quite comparable to aplastics in terms of antigen exposure, and the differential in HLA antibody formation that was observed was not related to a corresponding difference in the quantity of platelet administration (see Fig. 1).

Calculation of the frequency of transfusion revealed somewhat longer intertransfusion intervals in the leukemic group, but no apparent relationship between HLA antibody status and interval between platelet transfusions (see Table 6).

### DISCUSSION

Our studies have shown that alloimmunization to HLA antigens was not observed in the majority of our cancer patients. Only 27 of the 100 patients developed alloantibodies, and of that number, 14 reverted to an antibody negative status while supportive transfusion therapy was continued. Therefore, significant impairment of immune function as measured by the formation of antibodies to HLA antigens appeared to be present in these patients. Other investigators have documented the potential for marked immune impairment of leukemia patients undergoing chemotherapy and have shown that a greater deficit occurs in B-cell

### Table 5. Frequency of HLA Antibodies in Patients With HLA Alloimmunization on Repetitive Testing*

<table>
<thead>
<tr>
<th>Anti-HLA Antibody Frequency</th>
<th>Anti-HLA Antibody Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2 6</td>
<td>B12 4</td>
</tr>
<tr>
<td>A1 4</td>
<td>Bw35 4</td>
</tr>
<tr>
<td>A3 4</td>
<td>B5 2</td>
</tr>
<tr>
<td>A9 3</td>
<td>Bw21 2</td>
</tr>
<tr>
<td>A10 2</td>
<td></td>
</tr>
<tr>
<td>A11 2</td>
<td></td>
</tr>
<tr>
<td>Aew33 2</td>
<td></td>
</tr>
</tbody>
</table>

*Antibodies with frequency of occurrence of 2 or more.

### Table 6. Transfusion Duration and Mean Interval: One Transfusion is Assumed to be Equal to 4 U of Platelets

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Transfusion Duration (Mean)</th>
<th>Transfusion Interval (Days/Each 4U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute leukemia</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody positive</td>
<td>20</td>
<td>476 (days)</td>
<td>24</td>
</tr>
<tr>
<td>Antibody negative</td>
<td>45</td>
<td>214 (days)</td>
<td>11</td>
</tr>
<tr>
<td>Ewing's sarcoma</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody positive</td>
<td>2</td>
<td>296 (570,21)</td>
<td>8</td>
</tr>
<tr>
<td>Antibody negative</td>
<td>14</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody positive</td>
<td>5</td>
<td>29</td>
<td>3</td>
</tr>
<tr>
<td>Antibody negative</td>
<td>14</td>
<td>67</td>
<td>4</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody positive</td>
<td>7</td>
<td>44</td>
<td>4</td>
</tr>
<tr>
<td>Antibody negative</td>
<td>1</td>
<td>418</td>
<td>7</td>
</tr>
</tbody>
</table>

*For antibody positive represents number of units of platelets given until antibodies detected."
than in T-cell function. The current investigation extends these data to include the specific evidence for diminution of potential for alloimmunization associated with transfusion therapy. The group of solid tumor patients exhibited even more marked immunosuppression than did the leukemia patients, perhaps indicating that highly intensive chemotherapy or combined modality therapy has the potential for striking impairment of antibody formation, greatly minimizing the immunization resulting from transfused platelets.

Prophylactic granulocyte transfusion has been associated with a higher rate of alloimmunization than transfusion of platelets alone. In our patients, however, there seemed to be little influence of random donor granulocyte administration on HLA antibody formation. This might have been related to the fact that our patients were given granulocytes only for therapeutic purposes, i.e., to qualify for white cell administration, patients must have less than 500 granulocytes (and/or band forms) and a documented gram-negative bacteremia or (less commonly) progressive local infection in the face of adequate therapy, including drainage procedures where indicated. These characteristics were most commonly present during chemotherapy-induced periods of severe aplasia, when immune function was most likely to be maximally depressed. In our white cell recipients, the median interval from the initiation of transfusion support to the first administration of white cells was 18 mo. Clearly, then, these patients were not given granulocytes early in the course of their treatment. As will be discussed below, there is some evidence that HLA antibody development becomes progressively impaired in association with continued therapy, as well as data to show that this antibody activity, once present, may be lost over time despite repeated transfusions. These factors may, in part, explain the difference observed in the rate of alloimmunization related to white cell transfusions.

Tejada et al. reported in their study of nine leukemic patients that HLA antibodies, once present, decreased or disappeared over 88–193 days following the first transfusion to each patient. Although we did not document loss of antibodies in all alloimmunized patients, our data tend to confirm the development of either tolerance or suppression of immune response associated with continued therapy over time, in that 13 of our 25 patients developing antibodies reverted to an antibody negative status. These data serve to emphasize the importance of prolonged observation periods when evaluating alterations of immune function in patients undergoing intensive therapy for malignant disease. Short-term studies may overestimate the
probability of eventual alloimmunization when applied to patients whose treatment may be continued over extended periods.

It is possible that the changes in immune function may be due to more complex mechanisms than heretofore supposed. We believe that the low incidence of initial alloimmunization of our patients is due to the immunosuppressive character of chemotherapy (and radiation therapy in the Ewing's patients). The loss of HLA antibodies has been hypothesized as "possibly" due to continued immunosuppression secondary to ongoing intensive chemotherapy. However, this has been observed in patients with nonmalignant disease on dialysis, receiving repeated transfusions of packed red cells and whole blood. In that study, of 119 patients developing HLA antibodies, only 3% remained positive, despite continued "intermittent" transfusions to all; the authors likened these observations to similar occurrences in normal volunteers. It may be that factors such as dose of antigen, number and frequency of restimulation, and character of the antigen (e.g., antigenicity of different HLA antigens) may be fully as important as the state of the host immune system in determining whether HLA antibodies will persist once elicited.

It would appear, therefore, that in our 100 intensively treated cancer patients requiring high levels of platelet support, marked immunosuppression occurred, as measured by the ability to form antibodies to HLA antigens expressed on the surface of platelets. This failure to develop anti-HLA antibodies does not imply responsiveness to platelet transfusions; at present, we do not have sufficient data to comment meaningfully on the relationship between immunosuppression and posttransfusion platelet increment. It would, however, follow that in these types of patients, an assiduous search for potential causes of poor posttransfusion increments is indicated, and the assumption that alloimmunization is responsible requires confirmation by cytotoxicity assays for HLA antibodies. As increasingly intensive chemotherapy regimens and combined modality therapy become more common, this problem is likely to be encountered in a correspondingly higher proportion of patients. Although alloimmunization appears to occur in a comparatively limited proportion of such patients, those in whom it is present may require HLA-matched platelet support. It may therefore be more expedient to perform cytotoxicity assays on a regular basis, thereby enabling clinicians to select those patients actually in need of this costly and rare commodity. In addition, such screening procedures might aid in elucidating further information regarding the phenomenon of HLA antibody loss in the face of continued antigen exposure.

REFERENCES

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TV Holohan, PI Terasaki and AB Deisseroth