Characteristics and Natural History of Alloimmunization Following HLA-matched Leukocyte Transfusion in Hunter’s Syndrome

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ABSTRACT

The characteristics and natural history of alloimmunization to HLA were studied in five patients with Hunter’s syndrome receiving long term transfusions of leukocytes collected from human leukocyte antigen (HLA) matched donors. Patients were not given any other blood component transfusions.

All patients became alloimmunized at an average interval of eight months following an average of 15 transfusions. All patients developed HLA alloantibodies to transfused cross-reactive HLA antigens. Antibodies to transfused incompatible HLA antigens also developed in all patients. Multispecific HLA antibodies in which specificity determination could not be made were also seen in four patients.

In a small number of patients in this study, despite matching for the private HLA specificities, HLA alloimmunization was not prevented. In fact, broad alloimmunization was seen uniformly in our patients.

Introduction

Alloimmunization to human leukocyte antigens (HLA) can occur in any patient who receives a blood transfusion. In patients on hemodialysis and awaiting kidney transplant, alloimmunization is manifested by the production of oligospecific or multispecific HLA antibodies. Approximate 50 percent of polytransfused acute leukemia patients and up to 80 percent of polytransfused...
aplastic anemia patients also become alloimmunized. In the latter two conditions, multispecific antibodies are also commonly produced. In pregnancy induced alloimmunization, HLA antibodies should be theoretically restricted to the paternal incompatible HLA antigens. However, the antibodies produced in multiparous women are often multispecific. Even during deliberate immunization to procure monospecific HLA antibodies, multispecific antibodies are nonetheless seen. The hybridoma generated monoclonal xenoantisera more often define the major histocompatibility complex (MHC) determinants present in all the individuals (monomorphic) rather than more restricted determinants (polymorphic).

In clinical situations, transfusion support becomes difficult once the patient is alloimmunized. Kidney transplantation is also delayed if the humoral sensitization is extensive because finding a crossmatch compatible kidney becomes more difficult. In such circumstances, transfusion protocols designed to control or avoid alloimmunization would be desirable. Restricting the exposure to different donors has been tried in patients with acute leukemia with conflicting results. More recently, donor specific blood transfusion is being investigated in kidney transplantation. This latter approach has not been studied in other conditions. Also, attempts to minimize alloimmunization by transfusions of blood cells collected from HLA matched donors have not been studied. This phenomenon has been studied by the present authors in a group of patients with Hunter’s syndrome. In these patients, only the leukocyte concentrates collected with cell separators were transfused. No other blood components were given to these patients during the study period. Moreover, each patient was carefully followed with serologic studies to investigate alloimmunization. The rationale for transfusion of leukocytes in patients with Hunter’s syndrome is presented.

Hunter’s syndrome is caused by a deficiency of a lysosomal enzyme (α-L-idurono-2-sulfate sulfatase). Enzyme replacement by the transfusion of leukocytes has shown an increase in the catabolism of glycosaminoglycans, substances known to accumulate in various tissues in this disorder. Another approach to the enzyme replacement has been histocompatible fibroblast transplantation. Of these two approaches, leukocyte transfusions may be preferable because of the ease of collection and transfusion. This report describes our laboratory findings on alloimmunization. Clinical assessment of this form of treatment will be the subject of a later report.

Materials and Methods

HLA-A and B locus antigen typing was performed using a standard National Institutes of Health (NIH) microlymphocytotoxicity technique. Lymphocytes of normal blood donors were typed using 58 antisera defining 18 HLA-A locus specificities and 108 antisera defining 37 HLA-B locus specificities. The standard NIH microlymphocytotoxicity test was also used to test for HLA alloantibodies. The patient’s serum obtained at periodic intervals was screened against a panel of lymphocytes from 60 donors typed for most A and B locus antigen splits.

Serum was isolated from the patient sample within four to six hours of blood collection and stored frozen at $-65^\circ$C for less than one month prior to testing. The HLA antibody screening and specificity determinations were performed on each serum; absorptions to document further the assigned specificities were not performed. Serum samples showing cytotoxic reactions against greater than 15 percent of screening cells and with a reactivity pattern such that no specificity
could be assigned were considered multispecific. Selected serum samples from each patient were submitted to Immunohematology Laboratory of the American Red Cross Blood Services (Bethesda, MD) for testing. In each case, this independent laboratory was able to confirm our findings.

Time to alloimmunization was calculated in two ways once the transfusion therapy was initiated because of irregular intervals at which HLA antibody screening was performed. First, earliest possible time at which alloimmunization could have occurred was calculated based on the absence of HLA antibodies in the latest serum sample. Second, the observed onset of alloimmunization was calculated based on a serum sample which showed the presence of HLA antibody. It should be noted that the alloimmunization could have occurred between the time at which serum sample was negative and the one which showed the presence of HLA antibodies. This gap period for patients #1, #2, #3, and #5 was six, one, zero, and eight months, respectively. For patient #4, the first post-transfusion sample obtained at four months was positive and, therefore, this gap period could not be calculated.

Leukocyte concentrates for transfusion were collected using ACD-A as an anticoagulant. An eight cycle procedure was performed using 225 ml bowl at 60 to 70 ml blood flow rate. When the Buffy coat ring reached the shoulder of the bowl, the flow rate was reduced to 40 ml per min. The flow rate was further reduced to 15 ml per min when the ring had moved near the core of the bowl. During each cycle, after approximately 20 ml of Buffy coat rich plasma was collected, additional collection into the red cell layer was performed for precisely one minute. This Buffy coat rich product was subsequently centrifuged upright at 850 RPM for 7.5 minutes.† The supernatant platelet rich plasma was utilized for other patients and the leukocyte concentrates were suspended in a small volume of plasma and transfused to study patients. Our quality control data on 212 leukocyte concentrates prepared for transfusion indicate that the average (±SD) volume was 61 ± 20 ml with a hematocrit of 56 ± 16% containing 6.4 ± 2.2 × 10⁹ lymphocytes, 5.9 ± 2.1 × 10⁹ lymphocytes, and 5.5 ± 5.0 × 10¹⁰ platelets.

The HLA-matched leukocyte donors were selected among immediate relatives or unrelated blood donors based on a schema described by Duquesnoy et al. The degree of HLA-matching between the donor and the recipient was defined in the following manner. A match: the donor and recipient had identical phenotype; B1U: the donor lacked one of the antigens present in the recipient; B1XC: the donor deferred from the recipient by one cross-reactive antigen; B2U: the donor lacked two of the antigens present in the recipient; B2UX: the donor had two antigens that were identical with the recipient and the third donor antigen was cross-reactive with the recipient; B2X: the donor deferred from the recipient by two cross-reactive antigens; C: the donor had one antigen not present in the recipient and non-cross-reactive with the antigens of the recipient; D: the donor had two antigens not present in the recipient and non-cross-reactive with the antigens of the recipient; and R: the donor's phenotype was not known.

A computerized list of approximately 1,500 HLA typed donors was maintained. For each patient, approximately once a month, a computer search for

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† Sorval RC-3B centrifuge, using HG-4L rotor (120g), Du Pont Company, Biomedical Products Division Newtown, CT 06470.
HLA compatible donor was generated. The search identified the blood donors according to the match grades noted previously. The donor selection, when possible, was further based on the exclusion of antigens to which the recipient had already been immunized. Red cell compatibility and donor availability were other important criteria in donor selection.

An informed consent was obtained from parents of each study patient prior to initiation of transfusion therapy.

Results

A total of five patients with Hunter’s syndrome, confirmed by specific absence of α-L-idurono-2 sulfate sulfatase, were studied. Each patient received leukocyte concentrates collected from HLA matched donors from the initiation of transfusion therapy. Other blood products were not transfused. The frequency of transfusions was variable; in general, however, a daily transfusion was given initially for five days and one transfusion per month thereafter. All patients were males with an average age of 11 years (range two to 20 years) at the time of entry into the study (table I). On average, they were followed for 26 months (range 19 to 33 months) and had received 43 (range 22 to 58) leukocyte transfusions and were exposed to 31 (range 13 to 52) donors. Observed onset of alloimmunization to HLA antigens occurred at an average time interval of eight months and after an average of 15 leukocyte transfusions (table I).

Each transfusion was considered as representing four antigens of the donor (two antigens from HLA-A locus and two antigens from HLA-B locus) that could serve as immunogens to the recipient (table II). Thus, each one of the four antigens from the donor was classified into one of the four types of immunogen events. First, when a donor’s antigen was also present in the recipient, a compatible event occurred. Second, when the donor’s antigen was also present in the recipient, a compatible event occurred. Third, an incompatible event existed when the donor’s antigen was absent in the recipient. Fourth, an unknown event was present when the donor’s antigen was not known. Based on this form of analysis, a total of 856 events (total transfusions × 4) were found in five patients. Of these, there were 295 incompatible events. In the remaining 561 events, there were 277 compatible, 162 cross-reactive, and 122 unknown events.

Human leukocyte antigen matching grades between donor-recipient pairs are shown in table III. Approximately 50 percent of all pairs were either A or B matches. For an individual patient, the percentage of all matches which were A or B matched ranged from 27 percent to 74 percent.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Duration of Follow Up (mo)</th>
<th>Earliest Possible Alloimmunization (mo)</th>
<th>Observed Onset of Alloimmunization (mo)</th>
<th>Transfusions Before Observed Onset of Alloimmunization</th>
<th>Leukocyte Donors</th>
<th>Total Transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>8</td>
<td>33</td>
<td>7</td>
<td>13</td>
<td>27</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td>#2</td>
<td>20</td>
<td>27</td>
<td>5</td>
<td>6</td>
<td>19</td>
<td>52</td>
<td>57</td>
</tr>
<tr>
<td>#3</td>
<td>9</td>
<td>25</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>32</td>
<td>44</td>
</tr>
<tr>
<td>#4</td>
<td>2</td>
<td>19</td>
<td>NK*</td>
<td>4</td>
<td>10</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>#5</td>
<td>19</td>
<td>28</td>
<td>8</td>
<td>16</td>
<td>12</td>
<td>30</td>
<td>33</td>
</tr>
</tbody>
</table>

*NK = Not Known.
TABLE II

Immunogen Events in Patients Receiving Leukocytes from HLA-Matched Donors*

<table>
<thead>
<tr>
<th>HLA-A Locus</th>
<th>HLA-B Locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Compat-</td>
</tr>
<tr>
<td></td>
<td>ible</td>
</tr>
<tr>
<td>#1</td>
<td>63</td>
</tr>
<tr>
<td>#2</td>
<td>51</td>
</tr>
<tr>
<td>#3</td>
<td>28</td>
</tr>
<tr>
<td>#4</td>
<td>22</td>
</tr>
<tr>
<td>#5</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
</tr>
<tr>
<td>Percent</td>
<td>20.1</td>
</tr>
</tbody>
</table>

*The percentage of total immunogen events was based on a total of 856 events. See text for definitions of events.
†% of HLA immunogens.

A total of 104 screenings on the patients' serum samples were performed over a cumulative follow-up interval of 132 months (table IV). Therefore, the antibody screening occurred on an average of 1.3 months.

Although the number of incompatible events was higher than the cross-reactive events (295 vs 162), the specificities of antibodies formed in the recipients were directed largely at the cross-reactive antigens (tables II and IV).

ALLOIMMUNIZATION PROCESS

The nature of alloimmunization in each patient is shown in table IV and the details are presented as follows.

**Patient #1:** This patient manifested, cumulatively, antibodies against nine HLA antigens during the entire period of observation. The peak antibody response was seen at 22 months. The antibodies were directed predominantly against the HLA antigens of the B locus. One set of antibodies, namely anti-B7, BW22, B40, and BW41, was seen consistently. Other B locus antibodies were observed only transiently. The patient's HLA phenotype included HLA-B27 antigen, which is cross-reactive with HLA B7, BW22, B40, and BW41 antigens.17

The antibodies produced against HLA-A locus antigens had anti-AW24 and anti-A26 specificity. These antibodies were seen only transiently.

It is interesting to note that although the patient possessed HLA-A2 antigen, which has been shown to have a high degree of homology with HLA-B7 antigen,20 he nonetheless produced anti-B7.

**Patient #2:** Alloimmunization in this patient was first observed at six months of therapy. Peak antibody production occurred very soon thereafter; by the seventh month, his serum contained lymphocytotoxic antibodies against 90 percent of screening cells.

Although the patient possessed HLA-A2 antigen, he nonetheless manifested anti-A28 transiently. This occurred despite the fact that these two antigens are cross-reactive.17 Moreover, antibodies to the split antigens of HLA-A10, namely,
The data are a summary of serological findings noted with serum samples from each patient obtained during the entire follow-up period. The antibody specificities shown for each patient are cumulative specificities observed at different follow-up intervals.

Patient #4: In this patient, alloimmunization was first documented at four months. It reached a peak at ten months by which time the patient's serum was reactive against 89 percent of screening cells. The patient developed anti-A2 as well as anti-A9; both these antigens are cross-reactive with A3.\textsuperscript{17} The HLA phenotype of the patient indicated the presence of A3 antigen.

Patient #5: This patient demonstrated the longest pre-alloimmunization interval. Alloimmunization was not seen until 16 months after the start of treatment. However, the peak antibody production was evident by 20 months and was characterized by lymphocytotoxic antibodies against 92 percent of screening cells. An anti-A11 antibody was found in his serum although he possessed A1 antigen and even though A1 and A11 are cross-reactive antigens.\textsuperscript{17} Although the initial alloimmunization process in this patient was characterized by broad reactive HLA alloantibodies of undetermined specificity, antibodies of well defined specificities were seen later in the course of therapy. The loss of some antibodies produced earlier in the course of treatment may have been responsible for the ability to identify the specificities later in therapy.

Discussion

Despite our best effort at HLA matching of the donor-recipient pairs using a computerized list of approximately 1500 HLA typed donors and...
some family members, transfusion of incompatible donor HLA antigens appeared to be unavoidable. All patients became alloimmunized and in the absence of transfusions of other blood products, it appeared that leukocyte concentrates were responsible for immunizing the recipients. In view of previous studies, platelets were not considered responsible for the alloimmunization.\textsuperscript{3,10}

An analysis of antibody specificities revealed that the cross-reactive HLA antigens were frequently immunogenic. Although more B locus cross-reactive HLA antigens were transfused, the predominant antibodies to cross-reactive antigens were those towards A locus antigens. This raises an important question as to the immunogenic potential of the antigens from HLA-A and B loci: could it be that the antigens from A locus are more immunogenic? This question is difficult to resolve. Although it is believed that HLA antigens from A or B locus are equally immunogenic,\textsuperscript{17} others have indicated that the B locus antigens are more immunogenic than A locus antigens.\textsuperscript{18}

More importantly, the transfusion of platelets collected from donors mismatched for HLA cross-reactive antigen has been recommended for refractory patients.\textsuperscript{8} This is based on a postulate that cross-reactive donor HLA antigens should be compatible. However, in the present study, it was possible to demonstrate that alloimmunization to cross-reactive donor antigens was frequently seen. Previous documentation of immunogenicity of the cross-reactive antigens in a patient with aplastic anemia has been noted by us.\textsuperscript{13} This phenomenon has also been noted in kidney transplant recipients.\textsuperscript{15} In a recent report, two patients with aplastic anemia receiving platelet transfusions from donors selectively mismatched for cross-reactive HLA antigens also appear to have produced antibodies to transfused cross-reactive HLA antigens.\textsuperscript{16}

The implications of our findings reported here to other clinical situations wherein transfusion induced alloimmunization is important, such as in patients with acute leukemia and in kidney transplant patients, must be made with caution. This is particularly so because no assessment of the immune status of our patients was made by us. The patients with other disorders are frequently given chemotherapeutic agents and may be immunosuppressed. They may, therefore, not develop the alloimmunization in the same manner as our patients who did not receive any chemotherapeutic agents during the study period.

Besides the development of alloantibodies to HLA cross-reactive antigens, antibodies to other HLA antigens were also demonstrated. In addition, multispecific antibodies of undetermined specificity were also commonly observed. Also, in each patient, the first evidence of alloimmunization was characterized by one or more antibodies with well defined specificity (data not shown). Multispecific antibodies were found later in four out of five patients. This course of events suggests that multispecificity of the later antibodies may be due to the additive effect of sequential production of antibodies to mismatched private specificities. However, the possibility that multispecificity was due to a single antibody with broad reactivity cannot be ruled out. In fact, the reactivity pattern in some cases suggested that this may be the case. For example, patient #1 consistently manifested anti-B7, BW22, B40, and BW41. In this cross-reactive group, the private specificities appear to be located on a public determinant.\textsuperscript{22} Therefore, it is possible that this patient developed an antibody to a public determinant of this cross-reactive group and that the antibody was therefore found to react against each one of this family of private specificities. Even in kidney transplant recipients, antibodies to public determinants are felt to be impor-
tant. One proposed theory to explain allograft success in presence of HLA incompatibility is the possible matching for these public determinants or these “antigenic communities” between the donor and the recipient.5

Our studies on alloimmunization following HLA matched leukocyte transfusion in a small number of patients suggest that alloimmunization was not prevented. Alloimmunization was characterized by the production of antibodies to private HLA specificities including those mismatched but cross-reactive specificities. Multispecific antibodies were also commonly formed.

Acknowledgments

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