HLA Alloimmunization with Leukocyte Concentrates from HLA-matched and HLA-non-matched Donors in Patients with Hunter’s Syndrome*

RAM M. KAKAIYA, M.D.,†
ROBERT GREENSTEIN, M.D.,‡
PATRICIA PISCIOTTO, M.D.,§ SHEILA K. SLOCUM,†
DEBBYE ROSEN, R.N.,†
RITCHARD G. CABLE, M.D.,†
EDWARD E. MORSE, M.D.,‡ and
ANDREA GAINLEY, M.S.‡

†American Red Cross Blood Services
Connecticut Region

‡Department of Pediatrics and Division of Blood Bank
and Hematology,
Department of Laboratory Medicine,
University of Connecticut School of Medicine,
Farmington, CT 06032

ABSTRACT

The incidence and characteristics of HLA alloimmunization following transfusions of leukocyte concentrates as a source of enzyme replacement were determined in male patients with Hunter’s syndrome. Five patients were given leukocyte concentrates from HLA matched donors (Group I) and another five patients received leukocyte concentrates from non-HLA matched donors (Group II). No other blood products were transfused in either group.

Immune response pattern of HLA alloimmunization measured as the proportion of screening cells manifesting cytotoxicity with patient’s sera obtained during the follow-up period was similar in both groups.

HLA alloimmunization is seen with transfused leukocyte concentrates from either HLA-matched or non-HLA-matched donors in patients with Hunter’s syndrome. There appears to be a trend for an earlier onset of HLA alloimmunization with fewer transfusions when leukocyte concentrates from non-HLA-matched donors are transfused as compared to leukocyte concentrates from HLA-matched donors. Once HLA alloimmunization occurs, immune response patterns appear similar with either leukocyte product.

Introduction

Alloimmunization following blood transfusion occurs frequently. This phenomenon has been well studied for induction of alloantibodies to red cell antigens. The time course of alloimmunization to white cell antigens and the specificities of the antibodies produced after multiple transfusions has not been investigated in as much detail as is the...
case for alloimmunization to red cell antigens. The use of different transfusion protocols designed to modulate the alloimmunization process is increasing.\(^1\) Thus, understanding the nature of HLA alloimmunization has therapeutic relevance to a variety of clinical situations, such as kidney and bone marrow transplantation, as well as in cell supportive therapy for hematologic malignancies and enzyme replacement therapy in genetic metabolic diseases.

Our findings have been previously reported on the occurrence of HLA alloimmunization in patients receiving transfusions of HLA-matched leukocyte concentrates for enzyme replacement therapy in patients with Hunter's syndrome.\(^3\) The immune response was characterized by the production of a wide variety of HLA alloantibodies including monospecific, duospecific, and multispecific HLA antibodies, and antibodies with apparent HLA specificities to the crossreactive antigens of the recipients. In addition to the group of five patients in our previous study, HLA alloimmunization has been observed in five other patients with Hunter's syndrome.\(^3\) The time at which HLA alloimmunization occurred was calculated in two ways because the periodic screening of the serum for antibody was somewhat irregular. First, the earliest possible time at which alloimmunization could have occurred was based on the absence of antibodies in the latest serum sample. Second, the observed onset of alloimmunization was calculated based on a serum sample which showed the presence of lymphocytotoxicity observed with a serum sample against at least 15 percent of the panel cells.

The time at which HLA alloimmunization occurred was calculated in two ways because the periodic screening of the serum for antibody was somewhat irregular. First, the earliest possible time at which alloimmunization could have occurred was based on the absence of 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. An informed consent was obtained from parents of each study patient before transfusions of leukocyte concentrates were initiated. Leukocyte donors were selected based on a schema described by Duquesnoy et al\(^2\) for the first group of patients receiving leukocyte concentrates from HLA-matched donors. The

Materials and Methods

Lymphocyte HLA typing for the antigens of HLA-A and B loci was performed by the standard NIH microlymphocytotoxicity assay.\(^5\) HLA typing was performed 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 for HLA alloantibody screening and identification with a panel of 60 screening cells phenotyped for most HLA-A and B locus antigens, including the split antigens.
degree of HLA-matching between the donor and the recipient for all transfusions administered to this group I patients has been described in detail in our previous report. The second group of patients were provided leukocyte concentrates from non-HLA matched donors. In fact, in three of Group II patients, the HLA phenotype of the recipients was not determined. Both groups of patients received leukocytes from ABO compatible blood donors.

Leukocyte concentrates for transfusion to study patients were prepared as described by us previously and contained, on average, 6.1 ± 2.2 × 10⁹ (±SD) leukocytes. The lymphocyte and platelet content of this product was 5.9 ± 2.1 × 10⁹ and 5.5 ± 5.0 × 10¹⁰ (mean ± SD), respectively.

Statistical analysis was performed with a Mann-Whitney U test or a t-test.

Results

The characteristics of patients receiving either HLA matched (Group I) or non-HLA matched (Group II) leukocyte concentrates are shown in table 1. The range and distribution of ages in the two groups were similar. Since the duration of follow-up was less for the Group II as compared to Group I patients, they also received fewer transfusions. The average (±SD) frequency of all the transfusions was 18.2 ± 25.7 days (N = 214 transfusions) and 21.6 ± 30.3 days (N = 117 transfusions) for Group I and II patients, respectively. Therefore, transfusion frequency between the two groups was similar (p > 0.05). The transfusion frequency was also calculated for transfusions that preceded the observed onset of alloimmunization and gave an average (±SD) frequency of 13.4 ± 26.2 days (N = 75 transfusions) and 8.1 ± 12.4 days (N = 33 transfusions) for group I and II patients, respectively. This difference was statistically not significant (p > 0.05).

As described in the Methods section, the onset of HLA alloimmunization was calculated in two ways. First, the earliest possible time at which alloimmunization could have occurred was calculated

<table>
<thead>
<tr>
<th>Patient</th>
<th>Duration of Follow-up (mo)</th>
<th>Earlyest Possible Alloimmunization (mo)</th>
<th>Observed Onset of Alloimmunization (mo)</th>
<th>Transfusions Before Observed Onset of Alloimmunization</th>
<th>Total Transfusions</th>
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<tbody>
<tr>
<td>Group I</td>
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<td>3</td>
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<td>NK*</td>
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<td>15</td>
<td>28</td>
<td>8</td>
<td>16</td>
<td>12</td>
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<tr>
<td>Mean ± SD</td>
<td>11.4 ± 7.8</td>
<td>26.4 ± 5.1</td>
<td>8.4 ± 5.8</td>
<td>15 ± 8</td>
<td>42.8 ± 15.5</td>
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<tr>
<td>Group II</td>
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<tr>
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<td>18</td>
<td>30</td>
<td>1</td>
<td>1.5</td>
<td>6</td>
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<tr>
<td>7</td>
<td>14</td>
<td>8</td>
<td>NK*</td>
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<tr>
<td>Mean ± SD</td>
<td>8.1 ± 6.3</td>
<td>15.6 ± 9.4</td>
<td>2.7 ± 2.3</td>
<td>6.6 ± 3.0</td>
<td>23.4 ± 11.9</td>
</tr>
<tr>
<td>p</td>
<td>0.421</td>
<td>0.075</td>
<td>0.114</td>
<td>0.048</td>
<td>0.028</td>
</tr>
</tbody>
</table>

*NK = Not known
based on the absence of 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 one serum sample was negative and the following one which showed the presence of HLA antibodies. This time lag for group I patients, namely, 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 time lag could not be calculated. The time lag for group II patients, namely, patients #6, #8, and #9, was 0.5, one, and two months, respectively. For patients #7 and #10, the first follow-up sample obtained at five days and four months, respectively, was positive and, therefore, this time lag could not be calculated. As shown in table I, for Group II patients, the observed onset of alloimmunization was somewhat earlier and with fewer transfusions as compared to the Group I patients, $p = 0.048$ and 0.028, respectively.

All patients became alloimmunized. The immune response pattern for each patient was measured as the proportion of panel cells manifesting cytotoxicity with a given serum sample. As seen in figures 1 and 2, once the immune response was generated, it was generally well sustained in each patient. Only one patient, namely, patient #8, appeared to be a low responder as judged by a low percent reactivity with the patient's sera obtained at different intervals. This patient also received the least number of transfusions and was followed for the shortest interval (seven months). Except for this patient, the response pattern appeared similar among all other patients.

![Figure 1](image)

**Figure 1.** Patients #1 to 5 received leukocytes from HLA-matched donors. Percent cytotoxicity is the percentage of screening cells manifesting cytotoxicity with patient's serum.
**Discussion**

The findings demonstrate the occurrence of HLA alloimmunization to transfusions of non-HLA-matched leukocyte concentrates in patients with Hunter's syndrome. This is consistent with our previous observations in which HLA-alloimmunization was seen in a group of patients transfused with HLA-matched leukocyte concentrate. Donor-recipient HLA incompatibilities were not known for the group of patients who received non-HLA-matched leukocyte concentrates. In contrast, the donor-recipient HLA incompatibilities were clearly defined in patients receiving HLA-matched leukocyte concentrates. In fact, in these latter patients, as per our previous report, 12.7 percent of HLA-A locus and 21.7 percent of HLA-B locus donor antigens were incompatible with the recipient's HLA antigens. Even then, a trend towards an earlier onset of HLA alloimmunization with fewer transfusions was noticed with the non-HLA-matched leukocyte concentrates as compared to the HLA-matched leukocyte concentrates. This trend was not due to a difference in the age or in the frequency of transfusion between the two groups of patients. Eventually, all patients developed HLA alloimmunization regardless of the type of leukocyte product. Once alloimmunization was detected, the immune response pattern that followed was similar in both groups and was characterized by a consistent high percent lymphocytotoxicity found with the patient's sera during follow-up period.

It should be noted that the number of patients with Hunter's syndrome studied by us is small. Also, most of our patients were children. Thus, the implications of our findings to other situations in which
HLA alloimmunization occurs should consider the limitations mentioned in our study.

The data suggest that the immune response pattern is similar, once it occurs, regardless of whether HLA-matched or non-HLA-matched leukocyte concentrates are transfused. There appears to be a trend towards an earlier onset of HLA alloimmunization with fewer transfusions with non-HLA-matched as compared to HLA-matched leukocyte concentrates.

References


