Association Between HLA-D Region Antigens and Disease-Free Survival in Childhood Non-T, Non-B Acute Lymphocytic Leukemia

By James T. Casper, Marilyn Marrari, Vicki Piaskowski, Stephen J. Lauer, and Rene J. Duquesnoy

The frequency of three serologically defined HLA-D region antigens—DR, MB, and MT—was determined in a group of 74 children with non-T, non-B acute lymphocytic leukemia (ALL). Statistically, there were no significant differences in the frequency of any antigen in these ALL patients as compared with a panel of 85 normal controls. However, significant differences in HLA-DR frequencies were observed between patients who relapsed or who remained disease-free during a 30-mo period of chemotherapy. An increased incidence of relapse was associated with DR5, while disease-free remission during chemotherapy was associated with DR7. Life table analysis also demonstrated that DR5 was significantly associated with a decrease in disease-free survival in these patients. These data suggest that HLA-associated genetic factors may influence the responses of ALL patients to chemotherapy.

The possible association of HLA antigens with acute lymphocytic leukemia (ALL) has been a matter of controversy. Several reports have shown a weak association of certain HLA-A and B locus antigens (primarily A2 and B1), while other studies have shown no differences. Longer survival times have also been reported in ALL patients who typed for A2, and A9. It is therefore possible that HLA may play a role in the resistance and susceptibility to ALL.

In the mouse, susceptibility to virus-induced leukemia depends on the effects of a gene (Rgv-1) located in the I subregion of H-2. Other genes in this subregion control the immune response to certain antigens. Since HLA-D appears to be the human equivalent of the I subregion, it is possible that HLA-D region antigens may show stronger association with ALL than HLA-A and HLA-B antigens. De Moerloose et al. reported from cellular typing assays with HLA-D homozygous typing cells that the frequency of Dw7 was significantly increased in a group of 49 ALL patients (43% versus 12%). An increased incidence of DR7 was also found in a group of 31 children with ALL, but not in adult ALL. Most of these HLA-association studies are difficult to interpret because the heterogeneity among the ALL patient population was not assessed in terms of patient age, presence of cell surface markers, and disease-free survival.

The present study was designed to determine the frequency of HLA-D region antigens in patients with childhood ALL. Serologically, the HLA-D region consists of at least three defined systems: HLA-DR and two closely associated systems, MB and MT. In order to reduce the degree of heterogeneity of our patient population, we selected only patients who were less than 20 yr old and with non-T, non-B ALL. In a previous report, we described a transient appearance of HLA-DR-positive peripheral blood leukocytes in ALL patients after cessation of antileukemia therapy. An increased frequency of DR7 was found in a small group of 11 patients (55% versus 21% in normal controls). It was hypothesized that DR7 may be associated with an immune response gene that permits a better defense against the disease process or that DR7 predisposes to ALL. In an attempt to clarify these observations, we have extended our HLA-D region typing data to include a total of 74 ALL patients. Frequency patterns of DR, MB, and MT antigens were analyzed to determine possible association of these antigens with susceptibility to ALL, responsiveness to chemotherapy, and disease-free survival of these patients.

MATERIALS AND METHODS

Seventy-four patients with childhood ALL were typed for three HLA-D region antigens. Thirty-two patients were typed at the time of diagnosis. There were 37 males and 37 females with an age range of 0.5-19 yr (median: 4.5 yr). The median peripheral white cell count at diagnosis was 7,000, with a range of 1,500-418,000. Radiographically, none of the children presented with an anterior mediastinal mass. All patients were treated in a similar fashion with three-drug induction therapy (prednisone, vincristine, L-asparaginase), cranial irradiation plus intrathecal methotrexate, and maintenance therapy with 6-mercaptopurine and methotrexate alone or in combination with two other drugs (vincristine-prednisone, cytoxan-adriaycin, or cytoxan-cytosine arabinoside) for 30 mo.

Cell surface phenotypes of leukemic blasts of 61 children with ALL were determined at the time of diagnosis and/or relapse. Lymphoblasts from these children did not form spontaneous rosettes with sheep erythrocytes (E-rosettes) nor did they express T antigen(s) or cell surface immunoglobulins as determined in assays previously described. All patients expressed an antigen on their lymphoblasts that was detected by a heterologous antisera prepared against non-T, non-B ALL cells. The remaining 31 children were in remission before these assays for leukemic cell surface
markers were performed in our laboratory. However, because of their clinical presentation (median WBC: 6400, median age: 4 yr, absence of a mediastinal mass) and disease-free survival of >60 mo, these 13 children were included as non-T, non-B ALL patients.

Serologic typing for HLA-D region antigens was done by a long-term incubation (60 min with antiserum, 90-120 min with rabbit complement) lymphocytotoxicity test using a set of 72 or more B-cell alloantisera specific for DR1–DR7, MB1–MB3, and MT1–MT3. These sera were obtained locally from multiparous blood donors or through exchanges with other laboratories. The high quality of each serum was previously assessed in American and/or International Histocompatibility Workshops. Mononuclear cells were prepared from bone marrow and/or peripheral blood from patients (in remission and/or relapse) by velocity sedimentation over a Ficoll-Hypaque density gradient. When necessary, peripheral blood cells were enriched for B lymphocytes by removal of rosette-forming T cells using neuraminidase-treated sheep erythrocytes.

HLA-D region antigen frequencies were calculated for the 74 ALL patients and compared to 85 normal controls who were research personnel and/or blood donors between the ages of 17 and 60 without a history of hematologic abnormality. Differences in antigen frequencies were assessed for statistical significance using the chi-square test. The probability values (p values) for each of the 7 DR antigens were corrected by multiplying the calculated p value times 7. Cumulative proportions of patients with disease-free survival were calculated by life table methods. Differences in cumulative proportions were tested for statistical significance at 15, 30, and 45 mo by the z test for proportions. The significance of each overall curve and multivariate analysis was determined using Cox's regression model.

RESULTS

Table 1 shows the frequencies of HLA-D region antigens in the total group of 74 children and the control group of 85 normal healthy individuals. None of the HLA-DR, MB, or MT antigen frequencies showed significant differences between these groups of patients and controls. Furthermore, the distribution of HLA-D region antigens was similar in the 32 ALL patients typed at the time of diagnosis compared to the control population, suggesting we were not biasing the data in favor of a particular D-region phenotype.

Table 1. Frequencies of HLA-DR Antigens in Children With Non-T, Non-B ALL and Normal Blood Donors

<table>
<thead>
<tr>
<th>HLA Specificity</th>
<th>Leukemic Children* (n = 74) (%)</th>
<th>Controls (n = 85) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>DR2</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>DR3</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>DR4</td>
<td>26</td>
<td>35</td>
</tr>
<tr>
<td>DR5</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>DR6</td>
<td>14</td>
<td>27</td>
</tr>
<tr>
<td>DR7</td>
<td>31</td>
<td>26</td>
</tr>
<tr>
<td>MB1</td>
<td>56</td>
<td>60</td>
</tr>
<tr>
<td>MB2</td>
<td>48</td>
<td>39</td>
</tr>
<tr>
<td>MB3</td>
<td>44</td>
<td>52</td>
</tr>
<tr>
<td>MT1</td>
<td>53</td>
<td>60</td>
</tr>
<tr>
<td>MT2</td>
<td>61</td>
<td>62</td>
</tr>
<tr>
<td>MT3</td>
<td>44</td>
<td>54</td>
</tr>
</tbody>
</table>

*Seventy patients were typed for MB and MT determinants.

Fifty-three patients were studied during the 30-mo period of chemotherapy. Twenty-two patients were in remission at the completion of 30 mo of chemotherapy. It was found that the frequency of DR5 was increased in the group of ALL patients who relapsed on therapy but decreased in patients in continuous complete remission, p = 0.05 (data not shown). Conversely, DR7 was more prevalent in patients who were in remission at 30 mo than in patients who had exhibited relapses before cessation of chemotherapy (p = 0.1). None of the other DR, MB, or MT antigen frequencies showed significant deviations in these groups of patients. These data suggested that the presence of DR5 may be associated with higher incidence of leukemic relapses, while DR7-positive patients are likely to remain in continuous complete remission during the course of chemotherapy.

A possible association between DR5 and DR7 with disease-free survival of the total group of 74 ALL patients was further studied by life-table analysis. Figure 1 shows the proportion of patients in continuous complete remission as a function of time after diagnosis for 16 DR5-positive and 58 DR5-negative patients. Up to 15 mo, both groups responded equally well to chemotherapy with about 75% of patients being in remission. However, during the remaining time of observation (up to 72 mo), there was a significant decrease in disease-free survival of DR5-positive patients. For instance, 4 yr after diagnosis, the percentage of patients in continuous complete remission was 15% and 60% for DR5-positive and DR5-negative patients, respectively.

Figure 2 shows the results of a life table analysis of DR7-positive and DR7-negative patients. At 30 mo, the proportion of patients in continuous complete remission was significantly greater for the DR7-positive than for the DR7-negative group. After 30 mo the differences between these groups became less significant because several DR7-positive patients suffered a leukemic relapse during the year following cessation of chemotherapy. These data suggest that the possible association of DR7 with a prolonged remission during chemotherapy does not necessarily indicate a higher disease-free survival following cessation of chemotherapy.

Life table analysis was also done for groups of patients who were positive or negative for other DR, MB, and MT antigens. Only DR1-positive patients were doing significantly better at 15 mo (p < 0.02) but not at 30, 45, or 60 mo. No significant differences were found for any of the other groups.

The differences in disease-free survival between DR5- and DR7-positive patients may have been due to differences in clinical characteristics of these groups of
patients. However, no significant differences were found between the groups with regard to sex distribution, age, and white blood cell count at the time of diagnosis (Table 2). We further corrected the overall curves for DR5 and non-DR5 patients for age, WBC, and sex using the Cox model, and the difference remained significant at a \( p \) value of \(<0.03\).

**DISCUSSION**

These findings suggest that (1) there is no association between childhood non-T, non-B ALL with any HLA-D region antigen; (2) DR5 is associated with an increased incidence of relapses during chemotherapy and decreased disease-free survival time; and (3) DR7 is associated with a higher incidence of continuous complete remission during chemotherapy, but DR7 does not affect the overall disease-free survival after cessation of chemotherapy.

Childhood ALL is a heterogeneous disease in terms of clinical presentation (age, WBC) and biology (cell surface markers). Previous reports of HLA antigen frequencies in leukemic children have not always addressed the issue of biologic heterogeneity. Our

![Life table analysis of children with non-T, non-B ALL comparing DR5-positive (x—x) to DR5-negative (Δ—Δ) patients. Statistical analysis was performed at 15, 30, 45, and 60 mo. There was a significant difference at 30 and 60 mo (\( p < 0.02 \)).](attachment:image1)

![Life table analysis of children with non-T, non-B ALL comparing DR7-positive (x—x) to DR7-negative (Δ—Δ) patients. Statistical analysis was performed at 15, 30, 45, and 60 mo. There was a significant difference only at 30 mo (\( p = 0.05 \)).](attachment:image2)
patient population consisted of children with ALL whose lymphoblasts expressed an antigen characteristic of non-T, non-B ALL lymphoblasts but did not form E-rosettes nor possess T antigen or surface membrane immunoglobulins. Thus, patients with T-cell or B-cell ALL were excluded from the study.

Jeannet and coworkers have shown an excess of DR7 (and its cellular equivalent, Dw7) in children with ALL. The present data show that DR7 has a normal frequency in a total population of 74 children with ALL as well as in the group of 32 patients typed at the time of diagnosis. Our observations on ALL patients in continuous complete remission after chemotherapy may, however, be compatible with the data of Jeannet. Conversely, the frequency of DR5 was 9% in our patients in continuous complete remission after chemotherapy (versus 18% controls, see Table I).

Table 2. Comparison of Certain Clinical Parameters in DR5 and DR7 Patients With Non-B, Non-T ALL

<table>
<thead>
<tr>
<th>Sex</th>
<th>DR5 (n = 16)</th>
<th>DR7 (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>DR5 (n = 16)</th>
<th>DR7 (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>3</td>
<td>10/12</td>
</tr>
<tr>
<td>Range</td>
<td>9/12–17/12</td>
<td>1/12–14/12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>DR5 (n = 16)</th>
<th>DR7 (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>5,600</td>
<td>7,900</td>
</tr>
<tr>
<td>Range</td>
<td>2,200–119,600</td>
<td>1,500–418,000</td>
</tr>
</tbody>
</table>

No explanation for the HLA-DR effect described above is readily available. Because HLA-DR may correspond to a genetic region controlling immune responsiveness, it is possible that DR7 is associated with a gene that conveys an immune surveillance-like protection against leukemic relapse. Recently, De Bruyere and coworkers reported an association between HLA-A and B haplotypes and long-term survival in children with ALL treated with transfer factor. They speculate that this response may be secondary to the presence of a particular immune response (Ir) gene but did not type for DR antigens. For future studies, it may be important to assure an equal distribution of DR5 and DR7 patients receiving or not receiving transfer factor.

DR5 may predispose to a more aggressive form of non-B, non-T ALL, although a comparison of clinical data does not show any significant differences between DR5- and DR7-positive patients (Table 2). However, Bernard et al. recently described an increased incidence of DR5 (5 of 13) in children with Burkitt-type lymphoma, a particularly aggressive disease. Another consideration would be that leukemic cells from DR7-positive patients are more susceptible to chemotherapy than those from DR5-positive patients. Recent ALL treatment protocols have stratified patients to receive various chemotherapy on the basis of clinical presentation and lymphoblast phenotype. Conceivably, HLA-DR typing may be a helpful adjunct in this stratification process. It is possible that DR5-positive patients should receive a more aggressive chemotherapy regimen. On the other hand, DR7-positive patients are more likely to be in a remission during the 30 mo period of chemotherapy. However, a significant number of DR7-positive patients show leukemic relapses during the first year after cessation of chemotherapy. The question can be raised whether this group of patients should receive a longer course of chemotherapy. A prospectively controlled study will be needed to answer these questions.

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