ARROW TRANSPLANTATION from an HLA-matched sibling has been well established as a curative therapy for a variety of malignant and nonmalignant diseases.1-6 The establishment of volunteer donor registries has facilitated transplantation from unrelated donors for patients who lack a suitably matched related donor.7-13 Current standards for HLA typing include serological methods for class I HLA-A, HLA-B, and HLA-C antigens and DNA-based typing for class II HLA-DRB1 and HLA-DQB1 alleles. Molecular analysis has disclosed that the same serologically defined class I antigen can be encoded by an entire family of alleles. Among the 24 HLA-A, 50 HLA-B, and 11 HLA-C antigens, more than 86 HLA-A, 185 HLA-B, and 45 HLA-C alleles have been described.14 With sibling pairs, there is no need to identify the HLA class I and II alleles of the donor and recipient because they have inherited identical HLA haplotypes. With unrelated pairs, typing of HLA alleles is needed to evaluate genetic disparity, and matching of HLA alleles provides the closest possible approximation of the compatibility that can be achieved with a related donor.

Donor-recipient identity for HLA-DRB1 and DQB1 alleles reduces the risk of acute graft-versus-host disease (GVHD) and improves survival after unrelated marrow transplantation.13,15 However, with matching for serologically defined HLA-A and B antigens and for HLA-DRB1 and DQB1 alleles, the risks of acute GVHD, graft failure, and mortality remain higher with unrelated donors than with HLA-identical sibling donors. In this study, we tested the hypothesis that the increased risk of complications after unrelated marrow transplantation could be caused by mismatching for HLA-A, B, and C alleles of the donor and recipient. We found that outcome after unrelated donor transplantation can be optimized by matching the HLA-A, B, C, DRB1, and DQB1 alleles of the donor and recipient. However, mismatching for a single allele was well-tolerated. We also present evidence that class I and class II antigens have biologically different functions in clinical marrow transplantation. Whereas class I determinants govern graft acceptance, class II determinants play a role in GVHD.
A1/A2 primers (Lifecodes, Inc, Stamford, CT), and introns 1-3 of HLA-C were amplified with primer pair C1/C2 (Lifecodes, Inc). Amplified HLA-A and C templates were sequenced by using dye-labeled primers21,22 (HLA-A Sequencing Based Typing Kit; Applied Biosystems, Inc, Foster City, CA). In 133 typings, class I alleles were assigned by using oligonucleotide probe hybridization reagents (Lifecodes, Inc). Because most unrelated transplant pairs are HLA-DPB1 mismatched,23,24 no consideration was given to HLA-DPB1 matching in this study.

**Transplant procedure.** All patients were prepared for transplantation with intravenous cyclophosphamide (60 mg/kg recipient body weight) administered on each of 2 successive days followed by total body irradiation (1,200 to 1,575 cGy in 6 to 12 fractions) from dual opposed 60Co sources. Prophylaxis for CMV, fungal, and *Pneumocystis carinii* infection was administered as described.25,26 All protocols were reviewed and approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. Engraftment and the severity of acute GVHD were assessed according to criteria previously described.27,28

**Statistical methods.** The primary end point of this study was survival after transplantation. Grades III-IV acute GVHD and graft failure were secondary endpoints. Associations between type of donor and the hazard appropriate for survival and GVHD were examined by fitting multivariable proportional hazards regression models. Logistic regression was used to examine the probability of graft failure. Variables shown from previous studies to be associated with these end points were included in the multivariable models.13,20,27 Because the intention of this study was to assess associations between HLA allele matching and clinical outcome, parameters for age, gender, pretransplant therapy, stage of disease, body weight, and time interval from diagnosis to transplant are not displayed. Associations between these variables and clinical outcome have been reported elsewhere.

For analysis of each end point, donor and recipient pairs were categorized as follows: allele match at HLA-A, B, C, DRB1, and DQB1; mismatch involving only one class I allele; mismatch involving two or more class I alleles; mismatch involving only one class II allele; mismatch involving two or more class II alleles; and mismatch involving at least one class I allele and at least one class II allele. For analysis of GVHD, mismatching was defined as the presence of recipient alleles not shared by the donor (recipient disparity). For analysis of graft failure, mismatching was defined as the presence of donor alleles not shared by the recipient (donor disparity). For analysis of survival, mismatching included either type of disparity. The probability of GVHD was estimated as the cumulative incidence,29 with death, relapse, and graft failure without GVHD considered as competing risks. Kaplan-Meier estimates were used to describe survival.29 All P values resulting from regression models were derived from the Wald test.29,30

**RESULTS**

**HLA matching.** Of the 300 pairs in the study, 142 were matched for HLA-A, B, C, DRB1, DQB1 alleles. Among the 158 mismatched pairs, 83 were mismatched at only one HLA locus (21 HLA-A, 11 HLA-B, 26 HLA-C, 9 HLA-DRB1, and 16 HLA-DQB1) and 75 were mismatched at two or more loci. Eighty-three percent of the transplants were performed from a Caucasian donor for a Caucasian patient; the patient and donor were of the same race in 85% of the transplants.

**Graft failure.** Graft failure occurred most frequently with donors who were mismatched for more than one class I allele (Table 1). Graft failure did not occur with donors who were mismatched only at HLA-DRB1, DQB1, or both. After adjusting for marrow cell dose, the odds of graft failure was much higher with donors who were mismatched only for class I alleles than with matched donors (odds ratio, 10.5; 95% confidence interval, 2.2 to 49.8; \(P = .003\)). The odds of graft failure were also higher with donors who were mismatched both for class I and class II alleles than with matched donors (odds ratio, 10.0; 95% confidence interval, 1.7 to 58.4; \(P = .01\)). These results indicate that graft failure was caused primarily by class I disparities in the donor. We could not evaluate the role of individual class I loci, because the number of patients with graft failure was too small.

**Acute GVHD.** The risk of grades III-IV acute GVHD was influenced by the number and class of mismatched alleles in the recipient (Table 2 and Fig 1). Recipients with a single class I mismatch did not have an increased hazard of grades III-IV acute GVHD compared with matched recipients. Those with multiple class I mismatches appeared to have a higher hazard of severe GVHD than matched recipients, but the difference was not statistically significant. Recipients with both class I and class II mismatches had a significantly increased hazard compared with matched recipients. Recipients with a single class II mismatch also appeared to have a higher hazard than matched recipients. Although this difference was not statistically significant, the trend was suggestive, especially considering the relatively small number of patients. With respect to the development of grades III-IV acute GVHD, class II mismatches caused more difficulty than class I mismatches.

**Survival.** Patients with more than one class I mismatch and patients with both class I and class II mismatches had significantly lower survival than matched patients (Table 3 and Fig 2). In contrast, the survival of patients with a single class I or class II allele mismatch was similar to that of matched patients. Three of the seven patients mismatched for multiple class II alleles died before day 100. The other four remain alive from 3 to 8 years after transplant. These data demonstrate that a single class

<table>
<thead>
<tr>
<th>Donor Disparity</th>
<th>No. of Pairs</th>
<th>Graft Failures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele match</td>
<td>146</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Single class I</td>
<td>50</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Multiple class I</td>
<td>31</td>
<td>9 (29)</td>
</tr>
<tr>
<td>Single class II</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Multiple class II</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Class I and class II</td>
<td>34</td>
<td>4 (12)</td>
</tr>
</tbody>
</table>

*Seven patients had multiple class II mismatches, and 5 developed grades III-IV acute GVHD.

Table 1. Number of Graft Failures Among Patients Surviving to Day 28 According to Donor HLA Disparity

<table>
<thead>
<tr>
<th>Recipient Disparity*</th>
<th>No. of Pairs</th>
<th>Hazard Ratio (95% confidence interval)</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele match</td>
<td>146</td>
<td>1.0 (—)</td>
<td>—</td>
</tr>
<tr>
<td>Single class I</td>
<td>54</td>
<td>1.1 (0.7-1.9)</td>
<td>.66</td>
</tr>
<tr>
<td>Multiple class I</td>
<td>34</td>
<td>1.4 (0.8-2.6)</td>
<td>.27</td>
</tr>
<tr>
<td>Single class II</td>
<td>24</td>
<td>1.8 (1.0-3.4)</td>
<td>.06</td>
</tr>
<tr>
<td>Class I and class II</td>
<td>35</td>
<td>2.0 (1.1-3.4)</td>
<td>.02</td>
</tr>
</tbody>
</table>

Adjusted for stage of disease, body weight index, time interval from diagnosis to transplant, and pretransplant interferon therapy.

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I or class II mismatch is well tolerated, but multiple class I allele mismatches and simultaneous class I and class II mismatches should be avoided.

**DISCUSSION**

The results of this study demonstrate that survival after transplantation can be improved by matching HLA-A, B, C, DRB1, DQB1 alleles of the donor and recipient. The presence of multiple class I disparities in the donor was associated with an increased risk of graft failure, and the presence of class II disparities in the recipient was associated with an increased risk of GVHD. Disparity for a single class I or II allele did not appear to compromise survival. A parallel study of allele matching in a transplant population has been examined by the Japanese Marrow Donor Program.30

The presence of HLA class I disparity had important effects on the risk of graft failure, especially when two or more class I mismatches were present in the donor. The high risk of graft failure explains the reduced survival observed in this group of patients. The assessment of possible qualitative differences between class I loci must await a larger transplant experience because the numbers of donors with a single HLA-A, B, or C mismatch were too small for meaningful comparisons (21 HLA-A, 11 HLA-B, and 26 HLA-C).

The differences between class I and class II disparity in conferring risks of graft failure and acute GVHD reflect the distinct biologic functions of class I molecules and class II molecules.31,32 Class I molecules present peptides derived primarily from endogenously synthesized proteins, and the responding T cells express CD8. Class II molecules present peptides derived primarily from exogenously synthesized proteins, and the responding T cells express CD4. The peptides that bind respectively to class I and class II molecules differ in length and sequence.33 HLA class I molecules are further distinguished from class II molecules by their complex interaction with natural killer (NK) cells.34

**Table 3. Proportional Hazards Regression Model for Mortality According to Either Donor or Recipient Disparity**

<table>
<thead>
<tr>
<th>Donor or Recipient Mismatch*</th>
<th>No. of Pairs</th>
<th>Hazard Ratio (95% confidence interval)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele match</td>
<td>142</td>
<td>1.0 (—)</td>
<td>—</td>
</tr>
<tr>
<td>Single class I</td>
<td>55</td>
<td>1.2 (0.7-2.1)</td>
<td>.40</td>
</tr>
<tr>
<td>Multiple class I</td>
<td>35</td>
<td>3.5 (2.1-5.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Single class II</td>
<td>24</td>
<td>1.0 (0.5-2.1)</td>
<td>.89</td>
</tr>
<tr>
<td>Class I and class II</td>
<td>37</td>
<td>3.3 (2.0-5.5)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Seven patients had multiple class II mismatches and 3 died.

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**Fig 1. Cumulative incidence estimates of grades III-IV acute GVHD according to recipient disparity.**
Although complete allele matching is desirable, the presence of a single class I or II allele mismatch had little demonstrable effect on survival. These results are encouraging particularly for Africans, Asians, Hispanics, Native Americans, and other ethnic groups who have a low probability of finding a fully matched donor. Increased registry size and recruitment of specific ethnic populations can only partially alleviate this problem. Allowance for a single class I or II disparity offers access to marrow transplantation for patients who lack an HLA-A, B, C, DRB1, DQB1 allele-matched donor.

During the past decade, clinical laboratories have focused efforts on developing methods for prospective typing of HLA-DRB1 and DQB1 alleles in selecting donors for unrelated marrow transplantation. The results of our study provide further clinical rationale for efforts to develop methods for routine clinical typing of HLA class I alleles. The benefits of prospective class I allele typing will depend on the ability to identify optimally matched donors. Matching for HLA alleles may be more feasible for patients with CML than for patients with acute leukemia where progression of the disease might not allow an extended period of time for finding a donor. For patients with CML, results of transplantation are best when the time interval between diagnosis and transplantation is minimized, and a delay in transplantation introduced by a lengthy search for a donor could be detrimental.

Two further questions remain to be answered. First, we confined our current analysis to patients with CML, and the applicability of the results to patients with other diseases is unknown. For example, graft failure occurs very rarely in patients with acute leukemia and might not be associated with class I disparity. Second, the importance of matching for HLA-DPB1 alleles has not been defined. This question may be more answerable when the effects of class I disparity are better understood.

In unrelated marrow transplantation, efforts should be made to match HLA-A, B, and C alleles as well as HLA-DRB1 and DQB1 alleles of the donor and recipient. In patients with CML, the presence of a single HLA class I or class II disparity is well tolerated, but multiple disparities between the donor and recipient should be avoided wherever possible.

**ACKNOWLEDGMENT**

The authors are indebted to Dr Ji Pei and Leigh Ann Guthrie for sample procurement; Andrew Yamane, Mari Malkki, Mark Gatterman, and Brenda Nisperos for technical assistance; Amy Mellon for collection of clinical data; and Alison Sell for preparation of the manuscript.
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