Correlation Between Disparity for the Minor Histocompatibility Antigen HA-1 and the Development of Acute Graft-Versus-Host Disease After Allogeneic Marrow Transplantation

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Results of a previous study suggested that recipient mismatching for the minor histocompatibility antigen HA-1 is associated with acute graft-versus-host disease (GVHD) after allogeneic marrow transplantation. In that study, most patients received either cyclosporine or methotrexate for GVHD prophylaxis, and a cytotoxic T-cell clone was used to test for HA-1 disparity. To facilitate large-scale testing, we developed a method that uses genomic DNA to identify HA-1 alleles. A retrospective study was conducted to correlate HA-1 disparity and the occurrence of acute GVHD in 237 HLA-A2–positive white patients who had received a marrow or peripheral blood stem cell transplant from an HLA-identical sibling. All patients received both methotrexate and cyclosporine for GVHD prophylaxis. The presence of HLA-A*0201 was confirmed in 34 of the 36 HA-1 disparate pairs by sequencing the HLA-A locus. Grades II-IV GVHD occurred in 22 (64.7%) of these 34 patients, compared with 86 (42.8%) of the 201 patients without HA-1 disparity (odds ratio, 2.45; 95% confidence interval [CI], 1.15 to 5.23; \( P = .02 \)).

Recipient HA-1 disparity showed a trend for association with acute GVHD (odds ratio, 2.1; 95% CI, 0.91 to 4.68; \( P = .08 \)) when a multivariable logistic regression model was used to include additional risk factors. These data are consistent with results of the previous study, suggesting an association between HA-1 disparity and risk of acute GVHD, but the strength of this association may be lower in patients who received both methotrexate and cyclosporine alone than in those who received methotrexate or cyclosporine alone.

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MATERIALS AND METHODS

Patient selection. DNA samples were extracted from HLA-A2–positive white patients who received a marrow (n = 229) or peripheral blood stem cell (n = 8) transplant from an HLA-identical sibling at the Fred Hutchinson Cancer Research Center (Seattle, WA) between 1981 and 1996. Methods for HLA typing of donors and recipients have been described. All patients received methotrexate and cyclosporine for GVHD prophylaxis and had either grade 0 or grades II-IV acute GVHD. Patients with grade I GVHD, patients with renal failure requiring dialysis, and patients without GVHD who died before day 80 after transplantation were excluded. Because there were differences in GVHD grading according to the reviewers who assigned the grades before and after 1991, sample selection was designed to preserve a balance in the numbers of patients with grades 0 or II-IV GVHD before and after 1991. With evaluation of 200 donor/recipient pairs, a 50% incidence of grades II-IV GVHD, an 11.3% expected incidence of HA-1 disparity, and a 2-sided significance level of .05, the study was estimated to have 80% power to detect an odds ratio of 4.5 for the association between HA-1 disparity and grades II-IV GVHD.

HA-1 genotyping. Testing was performed by individuals who did not know whether the sample had come from a patient who had GVHD. Samples containing 100 ng DNA were subjected to 40 cycles of denaturation (94°C for 30 seconds), annealing (60°C for 30 seconds), and elongation (72°C for 60 seconds) using primers HA-1a and HA-1c, as described elsewhere. Five microliters of the amplified product was digested with 0.4 U Tsp54I or 0.5 U Fnu4HI (New England Biolabs, Beverly, MA) for 2 hours and then analyzed by electrophoresis in 2.2% agarose. When recipient HA-1 disparity was detected by analysis of restriction fragments, results were confirmed by allele-specific amplification. In this assay, samples containing 100 ng DNA were subjected to 40 cycles of denaturation (94°C for 30 seconds), annealing (60°C for 30 seconds), and elongation (72°C for 60 seconds) using primers HA-1a and HA-1c, as described above. Five microliters of the amplified product was digested with 0.4 U Tsp54I or 0.5 U Fnu4HI (New England Biolabs, Beverly, MA) for 2 hours and then analyzed by electrophoresis in 2.2% agarose.

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microliters of the amplified product was analyzed by electrophoresis in 2.2% agarose.

**Sequencing of HLA-A2.** The presence of HLA-A*0201 in HA-1 disparate pairs was confirmed by sequencing exon 2 and exon 3 of amplified HLA-A or HLA-A2 gene from the donor. Samples containing 100 ng DNA were subjected to 40 cycles of denaturation (94°C for 30 seconds), annealing (61°C for 30 seconds), and elongation (72°C for 90 seconds) using HLA-A2-specific 5’ primer A2F2M13R (5’-cagccagctagcatgaccTCTCAACCGACTCCTGCCCAGGCTCT-3’, positions 705-732, GenBank accession no. K02883; lower case letters indicate M13 forward primer). Alternatively, samples were subjected to 40 cycles of denaturation (94°C for 30 seconds), annealing (61°C for 30 seconds), and elongation (72°C for 90 seconds) using 5’ primer IN1CONSM13R (5’-cagccagctagcatgaccGTGAGTGCGGGGTCGGGA-3’, positions 599-616) and HLA-A-specific 3’ primer 18C182TM13F (5’-tgtaaaacgacggccagtGTGGC-3’, positions 1561-1540; lower case letters indicate M13 reverse primer) and HLA-A–specific 3’ primer AR1M13F (5’-tgtaaaacgacggccagtGGGAGAGTATCAGCCGGGTACG-3’, positions 1561-1540; lower case letters indicate M13 forward primer). The presence of HLA-A*0201 in HA-1 was detected in 36 of the 237 donor/recipient pairs (15.2%). HLA-A*0201 was present in 34 of the 36 donors. The other 2 had HLA-A*0205 instead of HLA-A*0201 and were excluded from further analysis. Twenty-two (64.7%) of the 34 patients with HA-1 disparity developed grades II-IV acute GVHD, compared with 86 (42.8%) of the 201 patients without HA-1 disparity (Table 1). In univariate analyses, recipient HA-1 disparity was significantly associated with an increased probability of grades II-IV GVHD (odds ratio, 2.1; 95% CI, 1.15 to 4.26; P = .02). The distribution of organ stages and overall grades of GVHD for patients with HA-1 disparity and those without HA-1 disparity. All P values are 2-sided, and no adjustments were made for multiple comparisons.

**RESULTS**

Recipient HA-1 disparity was detected in 36 of the 237 donor/recipient pairs (15.2%). HLA-A*0201 was present in 34 of the 36 donors. The other 2 had HLA-A*0205 instead of HLA-A*0201 and were excluded from further analysis. Twenty-two (64.7%) of the 34 patients with HA-1 disparity developed grades II-IV acute GVHD, compared with 86 (42.8%) of the 201 patients without HA-1 disparity (Table 1). In univariate analyses, recipient HA-1 disparity was significantly associated with an increased probability of grades II-IV GVHD (odds ratio, 2.1; 95% CI, 1.15 to 4.26; P = .02). The distribution of organ stages and overall grades of GVHD for patients with HA-1 disparity and those without HA-1 disparity. All P values are 2-sided, and no adjustments were made for multiple comparisons.

In a previous study of patients who received methotrexate and cyclosporine for prevention of GVHD, donor/recipient gender, donor parity, advanced malignancy, and total body irradiation (TBI) were identified as risk factors for grade II-IV GVHD. In the present study, patients with HA-1 disparity had more advanced malignancy than those without HA-1 disparity (Table 2), but the 2 groups were otherwise similar in risk factors for GVHD. In a multivariable logistic regression analysis, increased patient age and greater than 12 Gy TBI were significantly associated with higher risk of GVHD (Table 3). Other risk factors were not significantly associated with grades II-IV GVHD in this study population. With all factors included in the model, recipient HA-1 disparity showed a trend toward an increased probability of grades II-IV GVHD (odds ratio, 2.1; 95% CI, 0.91 to 4.68; P = .08).

**DISCUSSION**

Results of this study support the conclusion that recipient disparity for the HA-1 antigen is associated with an increased risk of GVHD, but the odds ratio in our study (2.1) is lower than the value reported by Goulmy et al7 (5.4). These results could reflect differences in methods used for sample selection, variability in GVHD grading, or the use of more effective GVHD prophylaxis in the population we selected for study. In the study by Goulmy et al,7 only 15% of the patients received both methotrexate and cyclosporine for GVHD prophylaxis, and all 10 adult patients with HA-1 disparity developed acute GVHD. In our study, all patients received both methotrexate and cyclosporine, and those who received reduced doses of cyclosporine because of acute renal failure were excluded. Twelve of the 34 patients (and 10 of the 31 more than 16 years of age) with HA-1 disparity did not develop GVHD. These data suggest that the combination of methotrexate and cyclosporine may have prevented the development of GVHD in some patients with HA-1 disparity.

Our finding that HA-1 disparity is associated with selectively

| Table 1. Correlation of HA-1 Disparity With Acute GVHD |
|---|---|---|
| **Skin Stage** | **HA-1 Disparity** | **Overall Grade** |
| | **Absent** | **Present** |
| 0 | 124 (62) | 14 (41) |
| 1 | 5 (2) | 3 (9) |
| 2 | 26 (13) | 2 (6) |
| 3 | 42 (21) | 15 (44) |
| 4 | 4 (2) | 0 |
| **Hair Stage** | **Absent** | **Present** |
| 0 | 154 (77) | 16 (47) |
| 1 | 29 (14) | 13 (38) |
| 2 | 8 (4) | 1 (3) |
| 3 | 5 (2) | 3 (9) |
| 4 | 5 (2) | 1 (3) |
| **Overall Grade** | **Absent** | **Present** |
| 0 | 115 (57) | 12 (35) |
| II | 53 (26) | 15 (44) |
| III | 29 (14) | 7 (21) |
| IV | 4 (2) | 0 |

Data indicate the n and (%) in each category. P values for differences in the distribution of skin, liver, and gut stages and overall grade were calculated from Wilcoxon rank sum tests.
increased severity of GVHD in the skin and gut but not the liver suggests that the tissue distribution of alloantigens in the recipient might influence the clinical manifestations of GVHD. Results of previous studies have suggested that the HA-1 antigen is expressed by hematopoietic cells, dendritic cells, and Langerhans cells but not by cultured fibroblasts, keratinocytes, or melanocytes. Further studies are needed to assess expression of the HA-1 antigen in vivo and to determine how the tissue distribution of this antigen is regulated.

Although the current data are consistent with the conclusion that HA-1 disparity is associated with increased risk of GVHD, the implications for hematopoietic cell transplantation remain to be determined. The availability of a simple DNA-based assay would make it feasible to type and match prospectively for HA-1 compatibility between the donor and recipient, but the opportunity for selecting among multiple HLA-identical related donors is low because of limited family size. Moreover, the prevalence of HLA-A2 among white patients is less than 50%, and the proportion of unselected recipients with HA-1 disparity is only 10% to 15%. With unrelated transplantation, multiple donors are available for patients who have common HLA haplotypes, and the proportion of unselected pairs with recipient HA-1 disparity is approximately 20% to 25%. On the other hand, the association between recipient HA-1 disparity and GVHD after unrelated marrow transplantation has yet to be demonstrated. Results of previous studies have suggested that the reduction in risk of GVHD from typing and matching for a single mHA is likely to be small, but substantial benefit could come from typing and matching for multiple mHA. Additional studies will be needed to assess the effects of HA-1 disparity on other important endpoints such as chronic GVHD, leukemia relapse, and survival.

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