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MISMATCHES OF MINOR HISTOCOMPATIBILITY ANTIGENS BETWEEN HLA-IDENTICAL DONORS AND RECIPIENTS AND THE DEVELOPMENT OF GRAFT-VERSUS-HOST DISEASE AFTER BONE MARROW TRANSPLANTATION

ELS GOULMY, PH.D., RONALD SCHIPPER, M.Sc., JOS POOL, ELS BLOKLAND, J.H. FREDERIK FALKENBURG, M.D., PH.D., JAAK VOSSEN, M.D., PH.D., ALOIS GRATWOHL, M.D., PH.D., GEORGIA B. VOGELSANG, M.D., PH.D., HANS C. VAN HOUWELINGEN, PH.D., AND JON J. VAN ROOD, M.D., PH.D.

Abstract Background. Graft-versus-host disease (GVHD) can be a major complication of allogeneic bone marrow transplantation even when the donor and recipient are siblings and share identical major histocompatibility antigens. The explanation may be a mismatch of minor histocompatibility antigens. We previously characterized five minor histocompatibility antigens, HA-1, 2, 3, 4, and 5, that are recognized by T cells in association with the major histocompatibility antigens HLA-A1 and A2.

Methods. We collected peripheral-blood leukocytes from 148 bone marrow recipients and their sibling donors, who were genotypically HLA identical. Fifty pairs were positive for HLA-A1, 117 were positive for HLA-A2, and 19 were positive for both. The pairs were typed with cytotoxic-T-cell clones specific for minor histocompatibility antigens HA-1, 2, 3, 4, and 5.

RECIPIENTS of allogeneic bone marrow grafts run the risk of graft-versus-host disease (GVHD) or graft failure, even when the donor and recipient have identical major histocompatibility antigens and are closely related.¹ These complications may arise from disparities in minor histocompatibility antigens between donor and recipient, with the antigen present in the recipient and not in the donor.^{2,3} In such cases, T cells in the transplanted donor marrow respond to minor histocompatibility antigens in the recipient.

Cytotoxic T lymphocytes directed against minor histocompatibility antigens of the host have been demonstrated in blood from recipients of bone marrow from donors who were genotypically HLA identical.⁴⁻¹⁰ Clones

Results. Mismatches of HA-3 were equally distributed among recipients in whom GVHD developed and those in whom it did not. By contrast, a mismatch of only HA-1 was significantly correlated with GVHD of grade II or higher (odds ratio, ∞ ; $P=0.02$) in adults. One or more mismatches of HA-1, 2, 4, and 5 were also significantly associated with GVHD (odds ratio, ∞ ; $P=0.006$) in adults. These associations were not observed in children.

Conclusions. A mismatch of minor histocompatibility antigen HA-1 can cause GVHD in adult recipients of allogeneic bone marrow from HLA-identical donors. Prospective HA-1 typing may improve donor selection and identify recipients who are at high risk for GVHD. (N Engl J Med 1996;334:281-5.)

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of such cytotoxic T cells have been isolated from lymphocyte populations in the blood of patients with severe GVHD. These clones have been used as reagents to identify five non-sex-linked minor histocompatibility antigens, designated HA-1, 2, 3, 4, and 5. Most of the cytotoxic-T-cell clones isolated from various patients reacted against HA-1.¹¹

For immune recognition, the HA-1, 2, 4, and 5 antigens must be presented to cytotoxic T cells by the major histocompatibility antigen HLA-A2. In this way they behave like antigens recognized in an HLA-restricted fashion. The HA-1 antigen is present in 69 percent of normal people who express HLA-A2, whereas the frequencies of the three others in this set of HLA-A2-restricted minor histocompatibility antigens are either high (95 percent for HA-2) or low (16 percent for HA-4 and 7 percent for HA-5). The HLA-A1-restricted minor histocompatibility antigen HA-3 occurs in 88 percent of persons positive for HLA-A1.¹¹ HA-1, 2, 4, and 5 are inherited independently of the HLA genes; each of them is a single gene, and none have a locus within the HLA region.¹²

We investigated whether mismatching of minor his-

From the Department of Immunohematology and Blood Bank (E.G., R.S., J.P., E.B.), the Department of Hematology (J.H.F.F.), the Department of Pediatrics (J.V.), the Department of Medical Statistics (H.C.H.), and Eurodonor Foundation (J.J.R.), Leiden University Hospital, Leiden, the Netherlands; the Division of Hematology, Department of Research, Kantonsspital, Basel, Switzerland (A.G.); and Johns Hopkins Oncology Center, Baltimore (G.B.V.). Address reprint requests to Dr. Goulmy at the Department of Immunohematology and Blood Bank, Leiden University Hospital, P.O. Box 9600, 2300 RC Leiden, the Netherlands.

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tocompatibility antigens contributes to acute GVHD (grade II or higher) in recipients of genotypically HLA-identical bone marrow. We collected peripheral-blood lymphocytes from 148 donor–recipient pairs of siblings who were positive for HLA-A1 or A2 and who were genotypically HLA identical. The lymphocytes from these donor–recipient pairs were analyzed by means of a series of cytotoxic-T-cell clones specific for five well-defined minor histocompatibility antigens, HA-1, 2, 3, 4, and 5.

METHODS

Patients

We studied 148 recipients of bone marrow and their sibling donors, who were genotypically HLA identical, at Leiden University Hospital, Leiden, the Netherlands; Kantonsspital, Basel, Switzerland; and Johns Hopkins Oncology Center, Baltimore. The donor–recipient pairs were selected on the basis of the presence of HLA-A1 or A2 (or both), which are the HLA restriction molecules for minor histocompatibility antigens HA-3 (HLA-A1) and HA-1, 2, 4, and 5 (HLA-A2).¹¹ Fifty pairs were positive for HLA-A1, 117 pairs were positive for HLA-A2, and 19 pairs were positive for both (Table 1). No other exclusion criteria were applied. There were 105 pairs of adults and 43 pairs of children (age, ≤ 16 years). The recipients underwent bone marrow transplantation between 1982 and 1990 for acute lymphocytic leukemia, acute myelocytic leukemia, chronic myelocytic leukemia, non-Hodgkin's lymphoma, or aplastic anemia. Consecutive patients were selected for the study. None of the recipients received bone marrow depleted of T cells. As prophylaxis against GVHD, they received methotrexate (51 patients), cyclosporine (80 patients), or both (17 patients); 6 patients also received prednisolone. In the assessment of GVHD, a grade of 0 or I was considered to indicate the absence of such disease, and a grade of II or higher its presence. All cases of chronic GVHD occurred in patients with grade II or higher acute GVHD. The relatively high frequency of GVHD in our study (60.7 percent) is most likely due to the use of methotrexate or cyclosporine as the principal form of prophylaxis.¹³

Blood Samples

Blood samples were obtained from the patients and their sibling donors before bone marrow transplantation and treated with heparin. The search for and selection of an HLA-identical sibling donor were based on HLA typing for the HLA-A, B, and DR antigens of the patients' families. Peripheral-blood leukocytes were isolated by Ficoll-Isopaque density-gradient centrifugation, washed, and resuspended in RPMI-1640 medium with 10 percent dimethyl sulfoxide for cryopreservation in liquid nitrogen.

Cytotoxic-T-Cell Clones Specific for HA-1, 2, 3, 4, and 5

The cytotoxic-T-cell clones specific for HA-1, 2, 3, 4, and 5 have been described in detail elsewhere.^{11,14} These clones were assayed for their ability to lyse phytohemagglutinin-stimulated peripheral-blood leukocytes from donors and recipients at various effector-to-target ratios. The leukocytes were labeled with chromium-51, and the extent of lysis was measured with a standard chromium-release assay.¹⁵ The assays were carried out retrospectively without knowledge of the clin-

Table 1. Status of Major Histocompatibility Antigens in 148 Donor–Recipient Pairs.

HLA-A1 STATUS	HLA-A2 STATUS		TOTAL
	+	–	
	no. of pairs		
+	19	31	50
–	98	0	98
Total	117	31	148

Table 2. Status of Minor Histocompatibility Antigens in 50 HLA-A1–Positive and 117 HLA-A2–Positive Donor–Recipient Pairs.*

HLA TYPE	NO. OF PAIRS	HA-1	HA-2	HA-3	HA-4	HA-5
HLA-A1	50	–	–	+	–	–
HLA-A2	117	+	+	–	+	+

*The HA-3 antigen is recognized in HLA-A1–positive persons, and the HA-1, 2, 4, and 5 antigens are recognized in HLA-A2–positive persons.

ical results. All experiments were repeated at least twice. A minor histocompatibility antigen was considered to be present when the percentage of lysis was at least 25 percent at the lowest effector-to-target ratio (i.e., 1 to 1).

Statistical Analysis

For this analysis of mismatching of minor histocompatibility antigens and GVHD, we considered a grade of II or higher as indicating the presence of GVHD.^{16,17} Each donor–recipient pair was categorized as matched or mismatched for each of the minor histocompatibility antigens. When the recipient was positive for the antigen and the donor was negative, the pair was counted as mismatched. Otherwise the pair was considered to be matched. We obtained maximum-likelihood estimates of the odds ratios for the association between match–mismatch status and GVHD, with stratification according to the age (adult or child) of the recipient. These ratios are presented with exact 95 percent confidence intervals and exact two-sided P values, calculated with the Egret statistical package.¹⁸ Heterogeneity between strata was evaluated with Zelen's exact test.¹⁹ Patients with missing values were excluded from the analysis of any of the minor histocompatibility antigens for which data were missing.

RESULTS

We typed 148 pairs of bone marrow donors and recipients who were genotypically HLA identical for HLA-A1 and A2 (Table 1). The HLA-A1–positive donor pairs were typed for HA-3, and the HLA-A2–positive pairs were typed for HA-1, 2, 4, and 5 (Table 2). Male recipients were considered to have H-Y, a sex-linked minor histocompatibility antigen, and were called H-Y–positive; female patients were considered to be H-Y–negative. The results were then evaluated to determine whether they were correlated with the development of GVHD after bone marrow transplantation.

We found no correlation of HA-3–antigen status with GVHD (Table 3). The HA-3–specific cytotoxic T cells we used to identify HA-3–positive donors and recipients were originally generated in a patient with severe acute GVHD. Nevertheless, the typing analysis in HLA-A1–positive pairs revealed an HA-3 mismatch in three patients with GVHD and four patients with no clinical signs of the disease (Table 3). We also noted this lack of correlation in our earlier studies.¹⁴

We found no influence of sex discordance in male (H-Y–positive) recipients of bone marrow from female (H-Y–negative) donors on the occurrence of GVHD. The H-Y antigen is influential in transplantation and can lead to graft rejection and GVHD.¹⁴ Among the HLA-A1–positive pairs mismatched for sex (male recipient, female donor), the number of recipients with GVHD was similar to the number without GVHD (six vs. five) (Table 3); similar patterns of distribution were

Table 3. Results of HA-3 Typing According to GVHD Status in 50 HLA-A1-Positive Donor-Recipient Pairs.

RESULTS OF TYPING (DONOR/RECIPIENT)*	HA-3		H-Y	
	NO		NO	
	GVHD	GVHD	GVHD	GVHD
	<i>no. of pairs</i>			
+/+	20	15	5	9
-/-	2	0	9	7
+/-	3	1	8	1
-/+	3	4	6	5
Not tested	0	2	0	0

*A plus sign indicates the presence of the antigen, and a minus sign its absence.

found in HLA-A2-positive male recipients of bone marrow from female donors (Table 4).

The effect of mismatches of HA-1, 2, 4, and 5 was studied in HLA-A2-positive pairs (Table 4). Since the numbers of mismatches for HA-2, 4, and 5 were small, we focused first on the effect of HA-1 mismatches. There was a significant association between an HA-1 mismatch and GVHD in adults (odds ratio, ∞ ; 95 percent confidence interval, 1.3 to ∞ ; $P=0.02$) but not in children (odds ratio, 1.2; 95 percent confidence interval, 0.02 to 26; $P=1.00$) (Table 5). Zelen's exact test (which measures heterogeneity between groups) showed no significant difference between the odds ratios in adults and children. The pooled odds ratio obtained from a stratified analysis was 5.4 (95 percent confidence interval, 1.0 to 56; $P=0.05$). It was notable that GVHD developed in all 10 cases in which the adult bone marrow recipient was HA-1-positive and the adult bone marrow donor was HA-1-negative (Table 5).

We also analyzed the effect of a mismatch of one or more of the minor histocompatibility antigens HA-1, 2, 4, and 5. We considered a mismatch to be present if the donor-recipient pairs were mismatched for at least one of these antigens. We considered a match to be present if the pairs were matched for all four antigens. Of the 115 HLA-A2-positive pairs tested for HA-1, 17 could not be classified because of missing information. The results for the remaining 98 pairs are shown in Table 6. For the group as a whole there was a significant association between a mismatch and GVHD (odds ratio, 6.4; 95 percent confidence interval, 1.4 to 43; $P=0.01$). The odds ratio in children was 1.9 (95 percent confidence interval, 0.1 to 22; $P=0.07$), and in adults it was infinite (95 percent confidence interval, 1.8 to ∞ ; $P=0.006$). Again, Zelen's test did not show a significant difference between the odds ratios in children and adults. The finding of a mismatch of more than one of the four antigens (HA-1, 2, 4, and 5) was slightly more predictive of GVHD than was the finding of an HA-1 mismatch alone (Table

5). GVHD developed in all 12 adult bone marrow recipients who were positive for at least one of the four antigens and whose donors were negative for those antigens.

DISCUSSION

Our study demonstrates a significant correlation between mismatches of minor histocompatibility antigens HA-1, 2, 4, and 5 in HLA-A2-positive donor-recipient pairs and GVHD. The frequency of the HLA-A2 phenotype is 49 percent in the white population. Among the 12 adult recipients with a mismatch of these antigens and GVHD, 8 had only an HA-1 mismatch; 1 had mismatches of HA-1 and 4, and 1 mismatches of HA-1 and 5; the 2 remaining recipients were matched for HA-1 but mismatched for HA-2 or 5.

The impact of mismatches of minor histocompatibility antigens on the development of GVHD is best studied in pairs of siblings who are genotypically HLA identical. In such pairs the effect of the disparity would not be overshadowed by unknown mismatches of major histocompatibility antigens. Siblings discordant for minor histocompatibility antigens are possible only in families in which both parents are heterozygotes or one parent is heterozygous and the other homozygous for the minor-histocompatibility-antigen allele.¹² A minor histocompatibility antigen is of clinical interest only if it is immunogenic and when it has a moderately frequent distribution in the population. Elkins et al.²⁰ failed to demonstrate any influence of mismatching of the minor histocompatibility antigen W1 on GVHD because the number of W1 mismatches was too low (i.e., there was a high phenotypic frequency). By contrast the HA-1 antigen fulfills two of the conditions required for the induction of GVHD: it is immunogenic and has a moderate phenotypic frequency (69 percent).

Other elements of the immunogenic potency of the HA-1 antigen must also be considered. For example, a response by cytotoxic-T-cell precursors that are specific for a minor histocompatibility antigen requires helper T cells. A large number of helper T cells, due to cross-reactivity or hyperactivation, might increase the frequency of cytotoxic-T-cell precursors specific for HA-1. Cross-reactivity of cytotoxic T cells themselves seems unlikely since, at least in vitro, the recognition of HA-1

Table 4. Results of HA-1, 2, 4, and 5 Typing and GVHD Status in 117 HLA-A2-Positive Donor-Recipient Pairs.

RESULTS OF TYPING (DONOR/RECIPIENT)*	HA-1		HA-2		HA-4		HA-5		H-Y	
	NO		NO		NO		NO			
	GVHD	GVHD	GVHD	GVHD	GVHD	GVHD	GVHD	GVHD	GVHD	GVHD
+/+	27	19	55	49	4	7	7	5	16	11
-/-	19	26	5	2	47	35	43	37	17	12
+/-	6	5	2	1	0	0	0	0	18	12
-/+	11	2	2	1	1	0	2	0	18	10
Not tested	1	1	0	0	12	11	12	11	2	1

*A plus sign indicates the presence of an antigen, and a minus sign its absence.

is governed solely by HLA-A2.1, and HA-1 segregates in families in a mendelian fashion.^{11,12}

The distribution of HA-1 in tissue might explain its correlation with GVHD. HA-1 and HA-2 are expressed only on cells derived from hematopoietic precursors, including dendritic cells and epidermal Langerhans' cells.^{21,22} Since the chief function of the latter cells is to present antigens to T cells, they are plausible candidates for eliciting a graft-versus-host reaction from donor T cells. The H-Y and HA-3 antigens occur on hematopoietic and nonhematopoietic cells. In parenchymal tissues of the host, they might induce immune tolerance in antihost cytotoxic T cells.²³ Such a mechanism can account for the absence of correlation between HA-3 and H-Y mismatches and GVHD. However, our results for H-Y are not in line with the report of an increased frequency of GVHD in male recipients of marrow from female donors.²⁴ This effect was seen primarily with female donors who had been pregnant or received a transfusion,²⁴ but it was not observed in all studies.²⁵

It has been suggested that GVHD is less frequent in young patients than in adult recipients of allogeneic bone marrow.^{24,25} Almost all of the children in our study were seen at the Department of Pediatrics at Leiden University Hospital, where the occurrence of GVHD is reduced because complete rather than selective decontamination of the intestinal tract is performed.²⁶ Our data are ambiguous with respect to the effect of mismatches of minor histocompatibility antigens on GVHD in children. The odds ratio of GVHD with a mismatch of HA-1, 2, 4, or 5 in this group was not significantly different from 1 (odds ratio, 1.9; 95 percent confidence interval, 0.1 to 22), but there was no significant difference between the odds ratio in children and the odds ratio in adults ($P=0.21$ by Zelen's test). This ambiguity is due to the small number ($n=34$) of children in the study.

In conclusion, our data demonstrate an association between the presence of an HA-1 mismatch and the occurrence of GVHD in adult recipients of bone marrow from genotypically HLA-identical donors. In all cases in which an HA-1-positive patient received bone marrow from an HA-1-negative donor, GVHD (grade II or higher) developed. Of 71 adult donor-recipient pairs

Table 5. Status of the HA-1 Antigen and GVHD in 115 Donor-Recipient Pairs.*

VARIABLE	CHILDREN		ADULTS		ALL PATIENTS	
	GVHD	NO GVHD	GVHD	NO GVHD	GVHD	NO GVHD
HA-1 status (no. of pairs)						
Mismatched	1	2	10	0	11	2
Matched	9	22	43	28	52	50
Total	10	24	53	28	63	52
Odds ratio	1.2		∞		5.4	
95% confidence interval	0.02-26		1.3-∞		1.0-56	
P value	1.00		0.02		0.05	

*Data on HA-1 status were missing for two pairs. The P value for heterogeneity between children and adults was 0.41.

Table 6. Status of the HA-1, 2, 4, and 5 Antigens and GVHD in 98 Donor-Recipient Pairs.*

VARIABLE	CHILDREN		ADULTS		ALL PATIENTS	
	GVHD	NO GVHD	GVHD	NO GVHD	GVHD	NO GVHD
HA-1, 2, 4, 5 status (no. of pairs)						
Mismatched	2	3	12	0	14	3
Matched	5	15	35	26	40	41
Total	7	18	47	26	54	44
Odds ratio	1.9		∞		6.4	
95% confidence interval	0.1-22		1.8-∞		1.4-43	
P value	0.07		0.006		0.01	

*Data were missing for 19 pairs. The P value for heterogeneity between children and adults was 0.21.

matched for HA-1, 28 of the recipients showed no clinical signs of GVHD. Our results suggest the clinical usefulness of HA-1 typing for HLA-A2-positive donor-recipient pairs to identify HLA-A2-positive recipients who are at high risk for GVHD. The HA-1 cytotoxic-T-cell clones that we used are available to others for typing. Molecular typing of these minor histocompatibility antigens may soon be feasible.^{27,28} The HA-2 antigen has already been identified and appears to originate from a member of the class I myosin family, a large family of proteins involved in cell locomotion and organelle transport.²⁷

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