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Incidence of humoral sensitization in HLA partially mismatched hematopoietic stem cell transplantation

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Abstract

As part of the 15th International Histocompatibility and Immunogenetics Workshop (IHIWS), seven centers participated in a collaborative project to determine whether any significant humoral sensitization occurred post-transplant among recipients of HLA partially mismatched hematopoietic cell transplants (HCTs). A total of 140 donor/recipient pairs were enrolled with a total of 367 pre- and post-transplant sera analyzed. The majority of the samples (69.1%) were obtained within 30–90 days post-HCT. HLA-specific antibodies were defined using single antigen bead assays on a Luminex™ platform with a positive cutoff value of 1000 normalized median fluorescence intensity (MFI). There was an overall incidence of post-HCT sensitization toward donor HLA mismatches of 5.7%; however, all cases were among recipients of one HLA haplotype-mismatched grafts under nonmyeloablative, pre-transplant conditioning. Among the one haplotype-mismatched recipients, 15.7% (8/51) developed donor HLA-specific antibodies and 29.4% also had antibodies directed toward third party HLA antigens. Among the donor-specific antibodies, 9.8% were directed toward HLA class I antigens; 7.8% were against class II antigens; and 2.0% had both class I and II specificity. The relative strength of post-transplant antibodies was low with no significant difference in the mean maximum MFI values between third party and donor-specific antibodies. Because only a small number (10.2%) of the post-transplant samples were obtained 180 days or more post-HCT, longer term study is needed to evaluate any clinical relevance of these low-to-moderate levels of donor-specific antibody in one haplotype-mismatched recipients, as well as to determine whether any other antibodies occur at later times.

Keywords

alloantibodies; hematopoietic cell transplants; human leukocyte antigen specific antibodies; human leukocyte antigen mismatched transplants; nonmyeloablative hematopoietic cell transplants

Introduction

In recent years, there has been increasing use of partially human leukocyte antigen (HLA) mismatched donors for hematopoietic stem cell transplantation (HCT), primarily because 30% or more of candidates lack a suitable HLA identical, related donor, but also, in some cases, in an attempt to gain a graft *vs* leukemia effect (1, 2). The degree of HLA mismatch may involve 1–2 alleles with related or unrelated, registry donors; multiple alleles with cord blood units; or a full HLA haplotype with related donors. The presence of donor HLA-specific antibodies (DSAs) prior to transplant is recognized to increase the risk of engraftment failure (3–4), which has led to the need for antibody analysis of candidates and either avoidance of donor mismatches to which patients have antibody or to reduce antibody levels through antibody depletion regimens (5–7). Less is known about the potential for the development of alloantibodies following HLA mismatched HCT. HLA-specific antibodies have been shown following allogeneic HCT from HLA matched donors, presumably from nonspecific activation of pre-existing memory cells or from transfusion support products (8–10). In an HLA mismatched transplant setting, humoral sensitization might arise either from host or donor origin and the related concerns would be increased risk of engraftment failure because of rejection or a possible contributing factor for chronic graft *vs* host disease (GVHD), respectively. Regardless of their origin, HLA-specific antibodies in sufficient titer could also contribute to patients becoming refractory to post-transplant platelet support (11).

The development of sensitive solid phase immunoassays that use purified HLA antigens as targets provides a means for the detection and specificity definition of very low levels of HLA-specific antibodies. Therefore, as part of the 15th International Histocompatibility and Immunogenetics Workshop (IHIWS), a collaborative study among seven centers was organized to investigate whether mismatched HCT results in any significant incidence of HLA sensitization.

Materials and methods

Study population

Contributing centers enrolled donors and recipients under appropriate institutional review board approvals. A total of 140 pairs were enrolled for whom a pre-transplant and at least one post-transplant recipient serum sample were available. Post-transplant sample intervals included 14 days, 30 days, 60–90 days, and 180 days or more, up to 1 year. Multiple post-transplant sera were available from 66 recipients, providing a total of 367 sera for analysis: 140 pre-transplant and 227 post-transplant. The majority (69.1%) of post-transplant samples were obtained within 30–90 days post-transplant. Samples at each interval, number and percentage, were as follows: 14 days-46 (20.3%); 30 days-92 (40.5%); 60–90 days-65 (28.6%); and 180 +days-24 (10.6%).

Characteristics of the study group are summarized in Table 1. Fifty-one (36.4%) of the recipients received grafts from one haplotype matched related donors with the remainder being comprised of one or more allele mismatched grafts from related (9.3%) or unrelated donors (54.3%). Forty (28.6%) recipients received multiple allele mismatched cord blood grafts (CBUs). The other stem cell sources included 38 (27.1%) mobilized peripheral blood

donors and 62 (44.3%) bone marrow donors. The majority of recipients were Caucasian (69.3%) and the gender distribution was 54.7% male and 45.3% female.

HLA-specific antibody analyses

Allele level HLA phenotypes were submitted for both donors and recipients which permitted analysis of post-transplant sera in both the forward and reverse directions. HLA-specific antibodies were identified using single antigen bead assays on a Luminex™ platform (One Lambda Inc., Canoga Park, CA). A positive cutoff was set at 1000 normalized median fluorescence intensity (MFI). Three centers performed their own antibody analyses ($n = 32$ recipients), but the majority were tested in the central laboratory (the Johns Hopkins University Immunogenetics Laboratory). Verification of all donor HLA-specific antibodies was performed in the central laboratory. Significance of differences in the mean, normalized MFI values of donor HLA-specific and third party HLA-specific antibodies were evaluated using a two tail Student's *t*-test after analysis for variance.

Results

Overall incidence of HLA-specific antibodies post-HCT

The overall incidence of HLA-specific antibodies directed against third party HLA antigens, i.e., nondonor and nonhost, detected among HCT recipient post-transplant was not significantly higher than the observed occurrence in pre-transplant samples. A subgroup of 121 recipient sera with complete antibody analyses were evaluated for HLA class I and class II specific antibodies pre- and post-HCT. The results are summarized in Figure 1. Increases, albeit not significant, were observed for both HLA class I and class II specific antibodies. The presence of class I specific antibodies increased from 27.3% of recipients pre-transplant to 29.7% post-transplant. Corresponding values for class II specific antibodies were 10.7%–14.9%.

Pre-transplant occurrence of antibodies in the forward direction against donor mismatched antigens was low, with only three cases observed (2.1%). Two of these were against HLA class I mismatched antigens and one was directed against a class II antigen mismatch. Only one of the class I donor-specific antibodies remained detectable post-transplant. The overall incidence of donor-specific antibodies was higher post-transplant with eight recipients (5.7%) having some donor-specific antibody. Notably, all of the donor HLA specific antibodies occurred among recipients of one haplotype-mismatched grafts. No donor-specific antibodies were detected among the allele mismatched recipients. Only one instance of apparent donor-derived sensitization against a host mismatched antigen was observed (0.7%) and that was directed against a DPB1 allele mismatch.

Incidence of post-HCT antibodies among one haplotype-mismatched recipients

Donor HLA-specific antibodies were detected among 15.7% (8/51) recipients of one haplotype mismatched donor grafts under nonmyeloablative conditioning protocols. An increase in antibodies specific for third party HLA antigens was also observed in 29.4% (15/51) of recipients. A comparison of the incidences of donor-directed and third party antibodies, with their respective class I and/or class II HLA specificity is given in Figure 2. For the donor-specific antibodies, 9.8% had specificity for HLA class I antigens and 7.8% for class II. Proportionately, a greater increase was noted among the third party antibodies with class I specificity than with the class I donor-specific antibodies: 27.5% compared with 9.8%. For antibodies with class II specificity, the proportions were identical, both being 7.8%.

Relative antibody strength

To evaluate whether there were any significant differences in the relative antibody strength post-HCT, the mean maximum MFI values of HLA donor-specific antibodies were compared with those of third party antibodies. For the donor-specific antibodies, the top ranking value corresponding to a donor mismatch was used, whereas for the third party antibodies, the value was simply the highest MFI for a given patient. These values are summarized in Table 2. The mean MFI values for both donor HLA-specific and third party directed antibodies were relatively low, the highest mean value being only 4196 ± 3109 MFI. Considered by HLA class I or II specificity, the mean MFI was higher for those antibodies with donor HLA class I specificity, but this difference was not significant. For class II specific antibodies, the mean MFI was lower among the donor-directed antibodies than with third party; however, again, this difference was not significant.

Discussion

The results from this collaborative study indicate that the overall post-transplant sensitization among recipients of partially mismatched hematopoietic stem cell grafts is low in the forward direction toward donor HLA mismatches (5.7%) and even lower in the reverse vector toward recipient mismatches (0.7%). However, donor-specific sensitization was frequent (15.7%) among the 51 recipients of a full HLA haplotype mismatch under pre-transplant nonmyeloablative conditioning. An increase in HLA-specific antibodies to third party antigens was also observed among 29.4% of the one haplotype-mismatched recipients. Post-transplant increases in HLA-specific antibodies toward third party antigens have been reported previously and may be attributable to post-transplant transfusion support (8,11). Among the cases in this study, the proportionally greater increase in HLA class I specific antibodies than for class II is consistent with post-transplant antigen stimulation from alloplatelet transfusions. Whether the donor-specific antibodies are occurring *de novo* or are evoked by post-transplant inflammation or transfusion is not clear. In all cases where donor-specific antibodies were found, the pre-transplant sera were additionally tested with the sensitive single antigen bead assays for any donor-specific antibody. Only one instance of pre-existing donor-specific antibody was confirmed. In all other cases, no pre-transplant donor-specific antibody was found that could have preceded the post-HCT antibody. These data suggest that sensitization is *de novo* post-HCT, but the possibility remains that some patients may have low frequencies of allo-specific memory B cells that are reactivated.

It is likely that differences in conditioning regimens may have contributed to the lower incidence of post-transplant HLA-specific antibodies among the recipients of allele mismatched allografts compared with the recipients of one HLA haplotype-mismatched grafts. Information on the conditioning regimens were available for 74 allele mismatched recipients and 51 haploidentical recipients. The conditioning regimens used varied from center to center and ranged from low intensity, nonmyeloablative to intense and fully ablative. However, all patients grafted with HLA-haploidentical, related donor marrow received truly nonmyeloablative conditioning, as autologous hematopoiesis recovered in nearly all patients experiencing graft failure (12). It is therefore possible that the higher incidence of donor-specific antibody after transplantation in this group was enabled by the persistence of host B cells after nonmyeloablative conditioning.

An important consideration of these findings is the relatively low levels of both donor-specific and third party antibodies that were observed. Using normalized MFI values as measures of relative antibody strength, no cases were found where the antibodies present were at levels consistent with positive reactions in a flow cytometric crossmatch (13). However, the post-transplant HLA class I donor-specific antibodies were found at levels that might be considered moderate and of potential risk (7). The primary goal of this project was

to determine whether there is any significant incidence of post-transplant sensitization resulting from HLA mismatched allografts. These results indicate that post-transplant sensitization is not infrequent with nonmyeloablative, haploidentical transplants, but the potential clinical relevance of the observed low-to-moderate antibody levels needs further investigation. Only limited and relatively short-term outcome data were available on 125 patients included in this study. As noted above, there was substantial variation in conditioning and post-transplant immunosuppression among the participating centers submitting allele mismatched transplants. Therefore, no meaningful analysis was possible with the small numbers of recipients in allele mismatched group. Outcome data are available on recipients of nonmyeloablative, haploidentical transplants with reported incidences of graft failure and acute grades 2–4 GVHD of 16% and 31%, respectively (14). Although the post-transplant development of donor HLA-specific antibody could certainly be a factor in graft failure, among nine haploidentical recipients in this trial who experienced graft failure, only three (33%) were found to have any detectable donor-specific antibody post-transplant (data not shown). Certainly, a larger trial among one haplotype-mismatched recipients is warranted to evaluate the potential clinical relevance of post-transplant antibodies. Furthermore, because only 10% of the total sera analyzed in this collaborative trial were obtained at 180 days or more post-HCT, longer term follow-up is needed to determine whether the donor reactive antibodies persist and/or whether additional HLA-specific antibodies may arise.

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Figure 1. Overall incidence of sensitization to human leukocyte antigen (HLA) third party antigens. The incidence pre- and post-hematopoietic cell transplant (HCT) is shown by HLA class I (open bars) or class II (cross-hatched bars) specificity.

Figure 2.

Post-hematopoietic cell transplant (HCT) human leukocyte antigen (HLA)-specific antibody incidence among recipients of one HLA haplotype-mismatched grafts. The percentages of one haplotype-mismatched recipients with HLA donor or third party specific antibodies are illustrated both pre- and post-HCT. Total antibody incidence is shown by white bars; HLA class I specific antibody incidence by light gray bars; HLA class II specific antibody with cross-hatched bars; and both class I and II incidence with black bars.

Table 1

Study group characteristics

	Asian	Black	Hispanic	Caucasian	Other
Recipient race/ethnicity	8(5.7) ^a	13(9.3)	5(3.6)	97(69.3)	17(12.1)
HLA mismatch	Allele mismatch				One-haplotype mismatch
	Related	Unrelated			
	13(9.3)	76(54.3)		51(36.4)	
HSC source	Cord blood	Mobilized peripheral blood		Bone marrow	
	40(28.6)	38(27.1)		62(44.3)	

HLA, human leukocyte antigen.

^aValues are given as the number in each category and the percentage in parentheses of the total 140 recipient group.

Table 2
Relative donor-specific and third party HLA-specific antibody strength among one haplotype-mismatched HSCT recipients

HLA antigen specificity	Class I specific antibody ^a	Class II specific antibody ^a
Donor specific	4196 ± 3109	1976 ± 1292
Third party	3040 ± 2416	3916 ± 2326
p ^b	0.422	0.256

HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; MFI, median fluorescence intensity.

^aValues are mean normalized MFIs ± standard deviation.

^bSignificance from two tail Student's *t*-test, assuming equal variance.