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Donor-specific anti-HLA antibodies in unrelated hematopoietic cell transplantation for non-malignant disorders

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To the Editor:

The role of pre-existing donor-specific anti-human leukocyte antigen antibodies (HLA-DSA) in hematopoietic cell transplantation (HCT) is the subject of much debate, reflecting the increasing feasibility of successful transplantation across HLA mismatch barriers. In our study of patients transplanted for non-malignant disease (NMD), HLA-mismatching increased the risk of graft failure and was 2 to 3 fold higher for the compared to patients with malignancies.[1] After adjustment for other factors, the odds ratio for primary or

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Authorship Contributions

AW, MFV, SJL, DT and SRS designed the study; GC performed HLA-DSA testing; TW and MDH performed statistical analyses; AW, MFV, SJL and SRS interpreted analysis, AW and SJL wrote the paper; TW, MDH, GC, KF, JH, KH, DT, MV, SRS and MFV critically reviewed and revised the paper.

Conflict of Interest

The authors have no conflict of interest to declare.

secondary graft failure for 7/8 and 6/8 matched pairs was 2.81 (1.74–4.54; p<0.0001) and 2.22 (1.26–3.97; p=0.006), respectively.

From the original study of 663 patients with NMD, we tested 236 patients with pre-transplant samples for HLA-DSA by solid phase assays utilizing single antigen bead preparations that included detection of IgG antibodies or by complement fixing antibodies based on the C1q binding assay.[2,3]. HLA-DSA was evaluated by analyzing the reactivity against the mismatched donor antigens determined by IgG or C1q assays; mean fluorescence intensity (MFI) >1,000 was considered positive, MFI >500 and <1,000 was considered potentially positive, and MFI< 500 was considered negative.

The primary outcome tested in the models was primary graft failure; the secondary outcome was overall survival. Donor engraftment was defined as >500/μl neutrophils with >5% donor-derived cells within marrow or peripheral blood cell subsets. The univariate and multivariate probabilities of graft failure and survival were evaluated for different cutoffs defining DSA positive. All variables were tested for the affirmation of the proportional hazards assumption, then stepwise forward selection with a threshold of p<0.05 for entry and exit. Center adjustment assumed random effects. Interactions were tested between the explanatory variables and other significant covariates, and none were significant at p<0.05. To adjust for multiple comparisons, p<0.01 was considered significant.

The median age of tested patients was 9 years old (range <1 to 53). Reduced intensity or nonmyeloablative conditioning was used in 48%, most of the patients were given marrow grafts (82%), and most were given either anti-T cell serotherapy (78% ATG, 2% Campath) and/or a T cell depleted graft (44%). The HLA-DSA-positive (MFI>1000) cohort was similar with respect to age at HCT, race, sex, type of NMD, Karnofsky/Lansky score, and year of HCT, however there was a slightly higher proportion of marrow recipients (95% vs 80%, p=.04) when compared to the HLA-DSA-negative cohort. The C1q positive group did not differ from the C1q negative group for these variables. Table 1a shows the distribution of HLA-DSA.

Table 1b shows the lack of association of HLA-DSA with graft failure and survival. Results were similar when HLA-DSA IgG positive and C1q positive (11.5%) were combined for analysis (data not shown). We then used an MFI>5000 as the cutoff value to define a positive HLA-DSA; however, results remained non-significant for an association with graft failure (data not shown).

Several studies have shown a positive HLA-DSA is a potent barrier to hematopoietic stem cell engraftment.[4–6] A number of factors might explain why HLA-DSA was not found to contribute independently to the risk for graft failure in patients with NMD in our study. These patients largely received marrow grafts and many received ex vivo T cell depleted grafts, both of which are associated with higher rates of graft rejection compared to recipients of T-replete PBSC.[7,8] Furthermore, reduced intensity conditioning regimens commonly were used. Except for patients with immune deficiencies, most other patients with NMD have stronger immune systems compared to patients with hematologic malignancies who have been treated with cytotoxic chemotherapy. Together these factors

form a milieu in which alloreactive host T cells persist after transplant and may not be counteracted by sufficient donor alloreactivity, leading to graft rejection. In such a setting, the addition of donor-recipient HLA mismatching would further strengthen host alloreactive responses. Previous sensitization of the host to mismatched donor HLA might not necessarily increase this already heightened reactivity. Finally, specific HLA-DSAs may have different potency but we lumped all positive tests together for analysis.

An alternative explanation for the findings is a lack of power to detect a significant difference. The number of patients that were available for this re-analysis was small and the number with HLA-DSA smaller, which may have reduced the power to detect an effect of HLA sensitization. Additionally, we were not able to examine some other hypotheses, such as class I vs. class II HLA-DSA, or whether there was an association with prior transfusions or disease categories. Therefore, our results should not be interpreted to mean that HLA-DSA testing is not relevant, simply that we were not able to detect a strong association in our cohort. Thus, until larger analyses confirm these results, efforts to optimize all potential factors that could improve engraftment should continue for patients with NMD.

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Table 1a.

Incidence and mean fluorescence intensity of positive and potentially positive anti donor HLA-specific antibodies (N=236)

	Positive – N (%)	**MFI mean (range)**	**Potentially positive – N(%)**	**MFI mean (range)**
IgG	10 (4.2%)	6451 (1032–13076)	16 (6.8%)	654 (518–909)
C1q	8 (3.4%)	7686 (1036–19673)	3 (1.3%)	836 (639–966)

Abbreviations: Immunoglobulin G (IgG); mean fluorescence intensity (MFI)

Table 1b.

Results of univariate and multivariate modeling testing the association of donor specific antibodies with various outcomes. Univariate estimates at 1 year, multivariate HR (95%) CI and p-values are shown.

Endpoints	HLA-DSA IgG Positive[1] / Potentially Positive[2] vs. Negative	HLA-DSA IgG Positive vs. Potentially Positive / Negative	C1q Positive[3] / Potentially Positive[4] vs. Negative	C1q Positive vs. Potentially Positive / Negative
Graft failure	13% vs. 12%, 0.75 (0.23–2.47), 0.63	10% vs. 12%, 0.72 (0.10–5.28), 0.75	18% vs. 12%, 1.42 (0.34–5.95), 0.63	25% vs 11%, 2.19 (0.52–9.17), 0.28
Overall survival	42% vs. 52%, 1.20 (0.70–2.05), 0.50	30% vs 52%, 1.34 (0.62–2.88), 0.45	27% vs 52%, 1.40 (0.68–2.88), 0.36	13% v. 59%, 2.07 (0.94–4.56), 0.071

GVHD, graft-versus-host disease; HLA-DSA, donor specific anti-HLA antibody

[1] IgG positive HLA-DSA: HLA-A=3, -B=1, -C=1, -DPB1=6 (MFI >1000)

[2] IgG potentially positive HLA-DSA: HLA-A=1, -B=1, -C=2, -DQB1=1, -DPB1=11 (MFI 500–1000)

[3] C1q positive HLA-DSA: HLA-A=4, -DPB1=4 (MFI >1000)

[4] C1q potentially positive HLA-DSA: HLA-C=1, -DPB1=2 (MFI 500–1000)