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Complement-Binding Donor-Specific Anti-HLA Antibodies and Risk of Primary Graft Failure in Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Detection of donor-specific anti-HLA antibodies (DSA) has been associated with graft rejection in all forms of transplantation. The mechanism by which DSA increase the risk of graft failure remains unclear. We hypothesized that complement-binding DSA are associated with engraftment failure in hematopoietic stem cell transplantation (HSCT) and analyzed 122 haploidentical transplant recipients tested prospectively for DSA. Retrospective analysis to detect C1q binding DSA (C1q+DSA) was performed on 22 allosensitized recipients. Twenty-two of 122 patients (18%) had DSA, 19 of which were women (86%). Seven patients with DSA (32%) rejected the graft. Median DSA level at transplant for patients who failed to engraft was 10,055 mean fluorescence intensity (MFI) versus 2065 MFI for those who engrafted ($P = .007$). Nine patients with DSA were C1q positive in the initial samples with median DSA levels of 15,279 MFI (range, 1554 to 28,615), compared with 7 C1q-negative patients with median DSA levels of 2471 MFI (range, 665 to 12,254) ($P = .016$). Of 9 patients who were C1q positive in the initial samples, 5 patients remained C1q positive at time of transplant (all with high DSA levels [median, 15,279; range, 6487 to 22,944]) and experienced engraftment failure, whereas 4 patients became C1q negative pretransplant and all engrafted the donor cells ($P = .008$). In conclusion, patients with high DSA levels (>5000 MFI) and complement-binding DSA antibodies (C1q positive) appear to be at much higher risk of primary graft failure. The presence of C1q+DSA should be assessed in allosensitized patients before HSCT. Reduction of C1q+DSA levels might prevent engraftment failure in HSCT.

INTRODUCTION

Allosensitization is a common problem in both solid organ and hematopoietic stem cell transplantation (HSCT) [1,2]. Approximately 50% of all patients requiring a transplant could become allosensitized and develop anti-HLA antibodies, and up to 30% of patients might have donor-specific anti-HLA antibodies (DSA) that pose a threat to organ rejection or graft failure (GF) in HSCT [3,4]. Our group initially showed that DSA are associated with primary GF in HSCT with mismatched donors [5,6]. Although a clear association between DSA and GF in HSCT has been subsequently demonstrated [7-11], the mechanism by which DSA may cause GF in HSCT remains unclear.

Activation of the complement cascade has been shown in allosensitized recipients of solid organ transplantation and has been suspected in animal models of HSCT [12,13]. The classical pathway of the complement cascade is activated

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when the antigen–antibody complex binds to C1q and initiates activation of other complement components, resulting in the formation of membrane attack complex, which in turn causes cell lysis with apoptosis and clearance of the targeted cells [14,15].

In HSCT, DSA that target donor HLA antigens present on the surface of hematopoietic progenitor cells, and antigen–antibody complexes that may bind to C1q, activate the complement cascade and cause destruction of the donor cells, resulting in allograft rejection. C1q assay was developed to detect C1q binding (complement binding or complement fixing DSA) in allosensitized recipients of solid organ transplants [16,17]; however, whether complement cascade activation represents a mechanism that mediates graft rejection in HSCT remains unclear. Here we hypothesized that complement-binding DSA might be associated with primary GF in HSCT and assessed the joint impact of DSA and C1q activation in a cohort of allosensitized recipients.

METHODS

Patients

One hundred twenty-two consecutive patients received HSCT at the University of Texas MD Anderson Cancer Center between September 2005 and September 2013, 21 (17%) with T cell depletion ($CD34^+$ selection) and 101 (83%) using a T cell–replete bone marrow graft and post-transplantation cyclophosphamide, tacrolimus, and mycophenolate for graft-versus-host disease prevention, as previously reported by us [18,19]. Patients were tested prospectively between 2008 and 2013, whereas a small number of patients (treated before 2008) were tested retrospectively for the presence of DSA in pretransplant specimens. Retrospective C1q assay was performed on banked serum samples in all patients with DSA.

DSA Testing

Pretransplant sera of all patients were tested prospectively for anti-HLA class I and class II antibodies using multianalyte bead assays performed on the Luminex platform (Luminex, Austin, TX), including LABScreen PRA, LABScreen mixed methods for screening. The binding level of donor-specific antibody was determined by the LABScreen single-antigen bead assay (One Lambda, part of Thermo Fisher Scientific, Canoga Park, CA) per manufacturer's instructions, and results were expressed as mean fluorescence intensity (MFI). Briefly, 5 μL of mixed beads, HLA class I and class II single-antigen beads, were added to 20 μL of sample serum and incubated for 30 minutes at room temperature in the dark with gentle shaking. After washing with wash buffer 3 times, 100 μL of goat anti-human IgG secondary antibody conjugated with R-phycoerythrin was added, and the samples were incubated in the dark for 30 minutes at room temperature. After washing 3 times, the samples were read on a Luminex-based LABScan 100 flow analyzer. Antibody specificity and binding level were analyzed and determined through HLA Visual or HLA Fusion software from the manufacturer.

C1q Assay

Complement-binding antibodies were detected retrospectively for patients with DSA using the C1q assay as reported by Chen et al. [16]. The complement component (C1q) bound by the antigen–antibody complex was detected with an R-phycoerythrin–labeled anti-C1q antibody. Fluorescence intensity was measured using a Luminex-based LABScan 100 flow analyzer. DSA specificity and binding level were determined by the C1qScreen assay per manufacturer's instructions (One Lambda). Briefly, 5 μL of human C1q and 5 μL of HLA class I and class II single-antigen beads were added to 5 μL of heat-inactivated sample serum and incubated for 20 minutes in the dark at room temperature, followed by adding 5 μL of R-phycoerythrin–labeled anti-C1q antibody and incubation for 20 minutes in the dark at room temperature. The samples were analyzed and the C1q binding specificity determined.

Treatment of Patients with DSA before Transplantation

Twelve patients with DSA (55%) received desensitization treatment before transplant with alternate day plasma exchange × 3 (1 to 1.5 × plasma volume), replaced with either fresh frozen plasma or with albumin, starting 1 week before admission for transplantation/beginning of conditioning chemotherapy, followed by 1 dose of 375 mg/m^2 rituximab (Rituxan) the next day after completion of plasma exchange, followed 1 day later by 1 dose of intravenous immunoglobulin (IVIG) 1 g/kg × 1 (PE/R/IVIG) [5]. In addition, 5 patients received a buffy coat infusion on day −1, prepared from the same haploidentical donor.

Buffy Coat Preparation

Bone marrow stem cell donors underwent 2 autologous phlebotomies within 30 days of the scheduled bone marrow harvest. On both occasions, 500 mL of whole blood was collected in citrate-phosphate-dextrose adenine collection bags. The buffy coat was prepared from the second phlebotomy performed 2 days before marrow stem cell infusion (day −2). The second autologous whole blood unit was separated by centrifugation within 8 hours of collection into packed RBCs and plasma containing the buffy coat layer. The plasma component then underwent a second centrifugation to separate the buffy coat. The final RBCs and buffy coat volume consisted of approximately 40 to 50 mL, which was then cross-matched, irradiated, and ready for infusion to the patient. All donors consented and were tested for infectious disease markers in accordance with the current American Association of Blood Banks and US Food and Drug Administration guidelines.

Statistical Methods

Summary statistics were computed for all patients and within specific subgroups. Categorical variables were summarized by frequencies and percentages and their associations assessed using either Fisher's exact test [20] or the Fisher-Freeman-Halton test [21]. Continuous variables were summarized by median and range (minimum, maximum) and their associations with categorical variables assessed using the Wilcoxon rank sum test [22]. GF was defined as the patient not engrafting, experiencing delayed engraftment beyond day 28 post-transplant, or neutrophil recovery with autologous reconstitution verified by chimerism. Associations between GF and patient covariates were assessed by fitting a Bayesian logistic regression model for the probability of GF as a function of numerical DSA value within each C1q status subgroup, type of pretransplant T cell depletion (T cell depleted versus T cell replete), age, gender, race, and diagnosis. A similar model, including only DSA value within each C1q status as covariates, was fit in the subgroup of 17 patients who received T cell depletion. In each fitted Bayesian model, the expression $\Pr(\beta > 0 \mid \text{Data})$ is the posterior probability that the coefficient of the associated variable is positive. Values either >.99 or <.01 may be interpreted as highly significant, values ranging from .95 to .99 or .01 to .05 may be interpreted as significant, and values ranging from .90 to .94 or .06 to .10 may be interpreted as moderately significant.

Overall survival (OS) was computed from date of HSCT to date of last known vital sign for those groups categorized at or before HSCT date. For GF status, which was assessed after the date of stem cell infusion, OS was determined using a landmark analysis where 1 month after HSCT was defined as the landmark time. Patients who did not experience engraftment within 1 month of HSCT were categorized as GF. Patients alive at the last follow-up date were administratively censored. Time to engraftment was computed from date of HSCT to date of engraftment or administratively censoring at either last follow-up date or date of delayed engraftment. The Kaplan-Meier method [23] was used to estimate unadjusted distributions of OS, and time to engraftment and the log-rank test [24] was used to assess differences between groups, where appropriate.

All statistical analyses were performed using SAS 9.3 for Windows (Copyright © 2011 by SAS Institute Inc., Cary, NC). All statistical tests used a significance level of 5%. No adjustments for multiple testing were made.

RESULTS

Baseline Characteristics of Transplant Recipients

Of 122 patients who received a haploidentical transplant and were tested for anti-HLA antibodies, 22 (18%) were found to have DSA in the initial samples. The median DSA level in the initial sample for all patients was 6040 MFI (range, 85 to 28,615), whereas the median DSA level at transplant was 4667 MFI (range, 614 to 22,944). Patient characteristics overall and by DSA status are summarized in Table 1. From the entire cohort of patients, 19 of 58 female patients (33%) were allosensitized versus 3 of 64 male patients (5%; $P < .0001$). Similarly, a much higher percentage of DSA-positive patients were women (86%) compared with those who were DSA negative (39%; $P < .0001$). In addition, women had higher DSA levels than men: median 7858 MFI versus 864 MFI, respectively ($P = .021$). No other significant differences were found between the DSA-positive and -negative groups except that a significantly higher percentage of patients with