Biol Blood Marrow Transplant 22 (2016) 292–299

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Donor Immunization Against Human Leukocyte Class II Antigens is a Risk Factor for Graft-versus-Host Disease

Florent Delbos [1], Walid Barhoumi [2], Ludovic Cabanne [2], Florence Beckerich [2], Christine Robin [2], Rabah Redjoul [2], Safae Astati [2], Andréa Toma [2], Cécile Pautas [2], Hélène Ansart-Pirenne [1], Catherine Cordonnier [2], Philippe Bierling [1,3], Sébastien Maury [2,3,4,*]

[1] *Etablissement Français du Sang, Ile de France, HLA Laboratory, Créteil, France*
[2] *Department of Hematology, AP-HP, Hôpital Henri Mondor, DHU Virus-Immunity-Cancer, Créteil, France*
[3] *IMRB, University Paris Est Créteil, INSERM U955, Créteil, France*
[4] *Center for Clinical Investigation in Biotherapy, Créteil, France*

Article history:
Received 29 June 2015
Accepted 25 September 2015

Key Words:
Anti-HLA antibodies
Graft-versus-host disease
Risk factor analysis

A B S T R A C T

The sensitization to HLA antigens is caused mainly by pregnancy and transfusions; however, anti-HLA antibodies also may be detected in nulliparous females and nontransfused males, and thus specifically in hematopoietic stem cell transplantation (HSCT) donors. In such cases, the impact on HSCT outcome is known only for platelet transfusion refractoriness. This study addresses the impact on graft-versus-host disease (GVHD) of anti-HLA antibodies detected in voluntary unrelated donors. Among 100 donor/recipient (D/R) pairs, 33 and 82 showed at least 1 HLA class I and class II mismatch, respectively. Because class II mismatches were more frequent, we focused our detection on anti-class II antibodies, using the Luminex assay. Among 82 HLA class II mismatched D/R pairs, 26 donors (32%) had at least 1 anti-HLA class II antibody detected in peripheral blood. Recipients of a graft from an anti-class II immunized donor had a higher cumulative incidence for a first episode of either acute or chronic GVHD (2- year cumulative incidence, 88% versus 67%; $P = .03$), which was confirmed in multivariate analysis (hazard ratio, 1.7; $P = .04$). In particular, according to the National Institutes of Health classification scheme, the cumulative incidence of chronic GVHD was higher in recipients of immunized donors (multivariate hazard ratio, 2.5; $P = .02$). Identifying specificities of anti-class II antibodies revealed that 13 of 26 alloimmunized donors had recipient-specific antibodies, directed mainly against mismatched HLA-DPB1 alleles. Donor-derived anti-HLA antibodies could be detected in recipients up to at least 6 months post-HSCT, supporting their association with chronic GVHD. Donor immunization against foreign HLA antigens is a new parameter to predict the occurrence of GVHD after HSCT from HLA-mismatched unrelated donors.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) has been established as a mode of curative therapy for hematologic disorders. The outcome strongly depends on selection of the optimum donor. For this, numerous criteria are taken into account, but the most important is an HLA-matched donor. HSCT from an HLA-mismatched donor is associated with an increased incidence of graft-versus-host disease (GVHD), delayed immune reconstitution, and subsequent associated morbidity and mortality [1-3].

HLA genes are clustered in 3 main regions, designated HLA class I (containing HLA-A, -B, and -C loci), class II (containing HLA-DR, -DQ, and -DP loci), and class III (containing genes coding for several proteins with immune functions). HLA class I and II genes are the most polymorphic genes of the human genome, with more than 12,000 alleles. The probability of identifying a 10/10 HLA-A, -B, -C, -DRB1, and -DQB1− matched voluntary unrelated donor (VUD) is estimated at 1 per 1 million donors. Moreover, owing to the lack of linkage disequilibrium between HLA-DPB1 and the rest of the extended HLA haplotype, approximately 86% of 10/10-matched unrelated donors are mismatched at the

Financial disclosure: See Acknowledgments on page 298.
* Correspondence and reprint requests: Pr. Sébastien Maury, Assistance Publique−Hôpitaux de Paris (AP-HP), Hôpital Henri Mondor, 51 avenue du Mal de Lattre de Tassigny, 94010 Créteil cedex, France.
E-mail address: sebastien.maury@hmn.aphp.fr (S. Maury).

http://dx.doi.org/10.1016/j.bbmt.2015.09.027
1083-8791/

HLA-DPB1 locus [4]. In this setting where the vast majority of donor/recipient (D/R) pairs share at least 1 mismatch at the HLA-DPB1 locus, alloimmunized donors or recipients may have specific anti-HLA antibodies against their counterpart HLA mismatch.

The sensitization to HLA antigens may be caused by pregnancy, transfusions, or previous grafts [5], which is the main reason why anti-HLA antibodies are frequently detected in patients with hematologic disorders [6]. It is well known that detection of donor-specific anti-HLA antibodies (DSAs) in recipients is associated with an increased risk of graft failure in solid organ transplantation [7,8]. Many reports also have indicated that the presence of DSAs may lead to graft failure in recipients of a graft from a VUD [9,10], a related haploidentical donor [11,12], or unrelated umbilical cord blood [13,14]. Thus, DSAs are routinely screened for to ensure optimal donor selection. The use of a modern solid-phase immunoassay based on Luminex multiplex assays, the most sensitive technique for detecting anti-HLA antibodies, has become the standard for such screening [15,16].

Interestingly, anti-HLA antibodies also may be detected in nulliparous females and nontransfused males [17,18], and thus specifically in HSCT donors. Apart from their role in induced platelet transfusion refractoriness [19-21], their impact on HSCT outcome has not yet been explored. Theoretically, recipient-specific anti-HLA antibodies (RSAs) might affect the development of GVHD [22]. The aim of the present study was to address the impact of anti-HLA antibodies detected in VUDs on GVHD occurrence. Toward this end, we performed a retrospective analysis over a 9-year period in our center. Because most D/R pairs were mismatched only for class II antigens (mainly HLA-DPB1), we focused our analysis on the impact of anti-HLA class II antibodies detected in donors.

METHODS

Study Population

Between January 2006 and May 2014, 141 patients underwent consecutive HSCT from a VUD at Henri-Mondor University Hospital in Créteil, France, a center accredited by the Joint Accreditation Committee of the European Society for Blood and Marrow Transplantation and the International Society for Cellular Therapy since 2005. Of these 141 patients, 107 had cryopreserved serum from donor for analysis of anti-HLA antibodies. Because our primary aim was to analyze correlations with GVHD occurrence, we excluded patients who experienced graft rejection within the first 6 months after HSCT ($n = 5$), as well as those who underwent a second HSCT ($n = 2$), leaving 100 patients for analysis. Fifty-eight donor–recipient (D/R) pairs (58%) were 10/10 allele-matched at HLA-A, -B, -C, -DRB1, and -DQB1 loci, and 42 (42%) had 1 mismatch (9/10) in 1 of these class I ($n = 38$) or class II ($n = 4$) loci. Cord blood transplants were not considered in this study, owing to the lack of any cryopreserved material from the donors in this setting.

Clinical data, including GVHD parameters, were prospectively entered into the Promise database, the registry of the European Group for Blood and Marrow Transplantation, and were updated annually for each patient. In this database, historical criteria based on time of onset are used to classify and grade GVHD [23,24], taking into account clinical signs, biopsy analysis results from at least 1 involved organ, and exclusion of other causes of cutaneous, digestive, or liver function abnormalities or other potential manifestations of GVHD. Acute grade II-IV GVHD and chronic extensive GVHD occurred in 59 patients (59%) and 36 patients (36%), respectively. A retrospective review of medical records according to the National Institutes of Health consensus criteria resulted in reclassification of 6 of the 36 patients with chronic GVHD as late acute GVHD [25,26].

For each patient, the type and severity of the underlying hematologic disease were used to assign a Disease Risk Index (DRI), as defined by Armand et al. [27] in large cohorts. As recommended based on our cohort size, we used the validated and refined 3-group DRI, in which very-high-risk and high-risk groups are merged [27].

All patients and donors signed an informed consent for registration in the Promise database and cryopreservation of biological material for research purposes. This study was approved by the Institutional Review Board of Henri-Mondor University Hospital.

HLA Analysis and Anti-HLA Antibody Detection

All D/R pairs were typed in high resolution for HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQB1, and -DPB1 loci by polymerase chain reaction (PCR) using sequence-specific oligonucleotides (One Lambda, Canoga Park, CA) [28] combined with PCR using sequence-specific primers (Olerup SSP, Stockholm, Sweden) [29], following the manufacturer's instructions. Since 2012, this analysis has been implemented by PCR sequence-based HLA typing (S4 Sequencing Kit; PROTRANS, Hockenheim, Germany) for HLA-A, -B, -C, and -DRB1 loci. Because their polymorphism is limited, HLA-DQA1 and HLA-DPA1 were not included in this study [30].

Donor serum collected before transplantation (<30 days) was screened for the presence of anti-HLA antibodies using the LABScreen assay (One Lambda) [31]. Fluorescence was measured with a flow analyzer (LABScan 100; Luminex, Austin, TX) (Figure 1). Results were recorded as the normalized background (NBG) ratio for each bead in the assay ($n = 12$ for class I and $n = 5$ for class II). The NBG ratio (with #N indicating the number of a given bead) was calculated as follows: [(sample-specific fluorescent value for bead #N)−(sample-specific fluorescence value for negative control bead)]/[(background negative control serum fluorescence value for bead #N)−(background negative control serum fluorescence value for negative control bead)]. We determined the threshold of positivity in a distinct cohort of 30 nontransfused male blood donors, using the mean value plus 2 SD of the distribution of NBG values. Accordingly, an NBG ratio of >3 for any multiantigen bead was considered a positive result. We also quantified a global anti-HLA immunization score, using the average of NBG ratios of the 5 class II multiantigen beads [32].

In cases of positive screening, anti-HLA antibody specificities were identified when at least 1 HLA mismatch existed in the graft-versus-host (GVH) direction, defined as a recipient allele not shared by the donor. We used single-antigen (SA) beads coated with purified recombinant HLA class II antigen (LABScreen Single Antigen; One Lambda). Only HLA alleles with a frequency of >1% in the Caucasian and African populations were screened. Results were recorded as the normalized mean fluorescence intensity (MFI) for each bead, after a negative control serum was tested (One Lambda). In donors, HLA antibodies were classified as positive if the MFI was >500, based on a previous report [10]. RSAs were defined as anti-HLA antibodies in donor serum directed against a respective recipient HLA-mismatched antigen. The HLA class II SA beads specific for HLA-DP and -DQ loci supported heterodimeric alpha/beta chain molecules. When multiple beads sharing the same beta alleles but different alpha alleles were positive, the beads associated with the highest MFI value were considered when determining the presence of RSAs. Antibodies targeting the alpha chain were systematically ruled out, because none of them recognized beads supporting a heterodimer including the same alpha chain.

Among the D/R pairs in our cohort, all but 2 HLA class II mismatches were represented on screening beads. In the 2 others, the SA bead coated with the specific mismatched-antigen bead (HLA-DPB1*10:01 and HLA*DPB1*17:01) was negative.

Monitoring of anti-HLA class II antibodies in patients undergoing HSCT from RSA-positive donors was performed at 3 time points: the day of transplantation (day 0) and day 90 ± 5 and day 180 ± 5 after HSCT. Here a higher MFI threshold of 1000 was used, to overcome possible antibody contamination by antithymocyte globulin (ATG) or fresh-frozen plasma. RSAs of donors were analyzed for complement (C1q) binding using an SA flow bead assay (C1qScreen; One Lambda) [33].

Statistical Analysis

The primary study endpoint was the cumulative incidence of GVHD according to donor anti-HLA immunization. We took into account the time to the first episode of GVHD, whether acute or chronic. Accordingly, cases reported either as acute grade II-IV or chronic extensive GVHD were considered each time they required systemic immunosuppressive therapy. When estimating the cumulative incidence of GVHD, we considered death without GVHD a competing risk using the cumulative incidence curves, then performed comparisons using the Gray test, and used the Fine and Gray model to estimate the subdistribution hazard ratio (HR). Because graft rejection was an exclusion criterion, it was not considered a competing event for GVHD occurrence.

We first assessed the risk factors for GVHD occurrence by univariate analysis. Candidate factors included recipient and donor sex; D/R sex mismatch; female donor and male recipient versus other combinations; cytomegalovirus (CMV) serostatus of D/R pairs (double-seronegative D/R pairs versus other combinations); severity of recipient's underlying disease as assessed by the revised DRI; use of total body irradiation (TBI) versus none; use of ATG in the conditioning regimen versus none; source of stem cells (bone marrow versus peripheral blood); number of HLA mismatches in