Increased presence of anti-HLA antibodies early after allogeneic granulocyte colony-stimulating factor–mobilized peripheral blood hematopoietic stem cell transplantation compared with bone marrow transplantation

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We have recently shown that the use of allogeneic granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood hematopoietic stem cell transplantation (PBHSCT), as compared with bone marrow transplantation (BMT), is associated with increased titers of antibodies (Abs) directed against red blood cell ABO antigens. To further evaluate the influence of a G-CSF–mobilized PBHSCT graft on alloimmune Ab responses, we examined the frequency of anti-HLA Abs after transplantation in the setting of the same randomized study, comparing PBHSCT with BMT in adults. Anti-HLA Ab presence was determined by complement-dependent cytotoxicity assay (CDC) and flow cytometry in the recipient before and 30 days after transplantation as well as in the donor before graft donation. The use of PBHSCT was significantly associated with increased detection of anti-HLA immunoglobulin G (IgG) Abs early after transplantation as evidenced by flow cytometry (11 of 24 versus 4 of 27 transplant recipients, P = .03) and, less so, by CDC (5 of 24 versus 1 of 27 transplant recipients, P = .09). The difference between PBHSCT and BMT was further heightened when analysis was restricted to anti-HLA IgG Ab–negative donor/recipient pairs. In such a setting, early anti-HLA Ab was never detected after BMT but was repeatedly detected after PBHSCT (flow cytometry, 6 of 18 versus 0 of 17 transplant recipients, P = .02; CDC, 4 of 23 versus 0 of 26 transplant recipients, P = .04). Importantly, the PBHSCT-associated increase in anti-HLA Ab detection was observed despite a reduction in the median number of platelet-transfusion episodes per patient in PBHSCT versus BM transplant recipients (3 platelet-transfusion episodes [range, 1-21] in PBHSCT group vs 6 platelet-transfusion episodes [range, 3-33] in the BMT group; P = .02). In conclusion, this study strongly suggests that G-CSF–mobilized PBHSCT results in an increased incidence of circulating anti-HLA Abs and further confirms that the use of such a graft alters alloimmune Ab responses.

Introduction

Use of peripheral blood stem cells after granulocyte colony-stimulating factor (G-CSF) mobilization for autologous or allogeneic hematopoietic stem cell (HSC) transplantation is being increasingly considered.1-11 We and others have demonstrated that the use of such a graft has a significant impact on the number and function of immune cells present in the graft as well as on posttransplantation immune reconstitution when compared with a bone marrow (BM) graft.8,12-17 In particular, we have recently shown that use of allogeneic G-CSF–mobilized peripheral blood HSC transplantation (PBHSCT) can alter an alloimmune antibody (Ab) response, as evidenced by increased titers of Ab directed against red blood cell (RBC) ABO antigen (Ag) after PBHSCT when compared with BM transplantation (BMT).16 Such an altered immunohematological reconstitution after transplantation can have profound clinical consequences, as evidenced by the occurrence of severe hemolysis after minor ABO-incompatible PBHSCT.18-23

To further evaluate the influence of a G-CSF–mobilized PBHSC graft on the occurrence of anti-HLA Abs after transplantation, we have taken advantage of the multicenter randomized phase III clinical trial comparing allogeneic BMT to allogeneic PBHSCT conducted by the Société Française de Greffe de Moelle et de Thérapie Cellulaire7 (Paris, France) to prospectively compare the frequency of anti-HLA Ab detection after allogeneic PBHSCT versus BMT.

Patients, materials, and methods

Patients and blood sample collection

Between June 1997 and June 1999, 127 patients were enrolled in a clinical multicenter phase III randomized study conducted by the Société Française de Greffe de Moelle et de Thérapie Cellulaire comparing allogeneic...
PBHSCT with BMT from an HLA-identical sibling donor. The protocol was approved by an ethical committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale of Marseille 2) and was conducted according to the Helsinki accords for human subject research. All procedures were performed after donors and recipients gave written informed consent. In June 1998, a prospective immunobiological evaluation was initiated for patients subsequently entered in the study. This evaluation included (1) assessment of the donor before G-CSF administration, fewer than 24 hours after the last G-CSF infusion (PBHSCT), and at the time of HSC graft harvest (BMT); and (2) assessment of the recipient before and after 30 days after transplantation. From June 1998 to June 1999, 51 of 71 consecutive randomized patients (67%) (PBHSCT, n = 24; BMT, n = 27) were included in the present study. Twenty patients were not included because of missing (nonharvested n = 19; unavailable n = 1) samples from the donor or the recipient before or after transplantation.

Clinical study
The clinical study design and results have been previously reported. The randomization was stratified by diagnosis and by center to minimize the variations resulting from different practices in terms of supportive care and graft-versus-host disease (GvHD) prophylaxis. Patient and recipient characteristics are given in Table 1. In the PBHSCT arm, donors received 10 μg/kg/d subcutaneous G-CSF (iligrastim) (Rhône-Poulenc-Rorer, Mont-rouge, France) for 5 days. On the fifth day (day −1 of transplantation), the first HSC harvest was performed by apheresis. If CD34+/H11001+ HSC were obtained from the BM donors before or at the time of BM harvest (BMT); and (2) assessment of the recipient before and at day 10 after the harvest (BMT). The anti-HLA Ab detection was performed by a cytofluorimetric assay (Flow-PRA) (One-Lambda) as previously described.24 Single-donor platelet concentrates (PCs) were administered to treat or prevent hemorrhage when blood platelet counts were below 20 × 109/L. All RBC-Cs and PCs were leuko-reduced by prestorage filtration. The number and time of administration of PCs and RBC-Cs were recorded for all recipients. Similarly, administration of intravenous polyvalent immunoglobulin (IVIG) was documented.

Biological samples
Serum samples (Vacutainer; Becton Dickinson, Le Pont de Claix, France) were obtained from the BM donors before or at the time of BM harvest (n = 27); from the PBHSCT donors before and/or after G-CSF mobilization (before the first apheresis) (n = 24); as well as from all recipients before the conditioning regimen and at day +30 after transplantation (n = 51). In addition, whenever possible, “long-term” serum samples were obtained from BM (n = 9) or PBHSCT (n = 9) recipients from 1 to 2 years after transplantation. Blood samples were shipped by overnight mail to the Etablissement Français du Sang Bourgogne-Franche-Comté (Besançon, France), and sera were immediately cryopreserved.

Anti-HLA Ab detection
The presence of anti-HLA Ab was determined by the classical Terasaki complement-dependent cytotoxicity assay (CDC) and by flow cytometry (Flow-PRA; One-Lambda, Canoga Park, CA). The latter method, recently developed, detects both anti-HLA class I and anti-HLA class II immunoglobulin G (IgG) Ab with a higher sensitivity as compared with CDC.

### Table 1. Donor and recipient characteristics

<table>
<thead>
<tr>
<th></th>
<th>PBHSCT group (N = 24)</th>
<th>BMT group (N = 27)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donors</td>
<td>33 (22-51)</td>
<td>33 (20-63)</td>
<td>.70</td>
</tr>
<tr>
<td>Recipients</td>
<td>35 (21-51)</td>
<td>34 (20-50)</td>
<td>.88</td>
</tr>
<tr>
<td>Sex mismatch, donor → recipient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male → female (%)</td>
<td>4 (17)</td>
<td>10 (37)</td>
<td>—</td>
</tr>
<tr>
<td>Donors (%)</td>
<td>9 (38)</td>
<td>9 (33)</td>
<td>.42</td>
</tr>
<tr>
<td>Nulliparous females</td>
<td>3 (13)</td>
<td>4 (15)</td>
<td>—</td>
</tr>
<tr>
<td>Parous females</td>
<td>8 (33)</td>
<td>4 (15)</td>
<td>—</td>
</tr>
<tr>
<td>Males</td>
<td>13 (54)</td>
<td>19 (70)</td>
<td>.33</td>
</tr>
<tr>
<td>Diagnosis (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>10 (42)</td>
<td>11 (41)</td>
<td>—</td>
</tr>
<tr>
<td>ALL</td>
<td>3 (12)</td>
<td>6 (22)</td>
<td>—</td>
</tr>
<tr>
<td>CML</td>
<td>11 (46)</td>
<td>10 (37)</td>
<td>.70</td>
</tr>
<tr>
<td>Conditioning regimen (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBI-EDX</td>
<td>17 (71)</td>
<td>15 (56)</td>
<td>—</td>
</tr>
<tr>
<td>BUS-EDX</td>
<td>6 (25)</td>
<td>6 (22)</td>
<td>—</td>
</tr>
<tr>
<td>VP16-EDX-TBI + TAME</td>
<td>1 (4)</td>
<td>6 (22)</td>
<td>.18</td>
</tr>
</tbody>
</table>

AML indicates acute myeloid leukemia; ALL, acute lymphoid leukemia; CML, chronic myeloid leukemia; TBI, total body irradiation (median, 12 Gy; range, 11–13.5); EDX, cyclophosphamide (120 mg/kg); BUS, busulfan (16 mg/kg orally); VP16, etoposide (20 mg/kg); and TAME, tri-aracynite (12 g/m²) plus melphalan (140 mg/m²) plus etoposide (60 mg/kg).

The CDC assays were performed as previously described and involved the testing of serum samples against a panel of previously cryopreserved peripheral blood mononuclear cells from 30 HLA-typed donors. Serum samples were considered positive for anti-HLA Ab if they reproducibly caused at least 60% cytotoxicity in 1 or more cell populations or at least 40% cytotoxicity in 2 or more cell populations of the panel. In such cases, positivity was confirmed on a second panel of 30 different HLA-typed donors and tested in the presence or absence of dithiothreitol (DTT). Anti-HLA IgG Ab positivity persisted despite treatment with DTT, while the presence of IgM Abs resulted in no cytotoxicity in the presence of DTT. Panel reactivity was calculated as a percentage of the number of reactive cell populations among the panel. Because the serum samples were also tested by flow cytometry with the use of a method that allows a clear distinction between anti–HLA class I and anti–HLA class II IgG Abs, CDC testing against purified HLA-typed B cells after absorption on platelets was not performed.

Flow-PRA (One-Lambda) assays were performed according to the supplier’s recommendations. Briefly, 20 μL test serum was incubated for 30 minutes with 5 μL class I and 5 μL class II beads at 22°C. The beads were then washed twice with buffer and incubated for 30 minutes with 100 μL fluorescein isothiocyanate–conjugated goat anti–human IgG at 22°C in the dark. After 2 additional washings, the beads were fixed in 1% paraformaldehyde–hydroxide buffer. In each assay, pooled AB–sera known to be devoid of anti–HLA Ab (checked by CDC and flow cytometry on 3 different populations) was used as a negative control, whereas pooled sera containing polyspecific anti–HLA Ab was used as a positive control. Analysis was done with a FACScan (Becton Dickinson) on a 1024-channel linear scale. In the assays performed to determine a standardized cutoff value for class I or class II beads, tests were performed with a negative-control serum pool, and the threshold fluorescence intensity limit was arbitrarily set at the end of the peak. With such a threshold, 5% of the beads in the presence of the negative-control serum pool were found to be positive.

Detection of rheumatoid factors
Quantitative determination of serum rheumatoid factor (RF) was performed by nephelometry and hemagglutination. Nephelometry measured the end-point aggregation of small latex particles coated with human IgG (N Latex RF kit and BNII nephelometer; Dade Behring, Marburg, Germany). The
microplate hemagglutination technique (Laboratoires Fumouze, diagnostics division, Levallois-Peret, France) was based on the principle of the Waaler-Rose reaction and used sheep erythrocytes sensitized by the IgG fraction of rabbit anti–sheep erythrocyte serum. That technique discriminates RF and nonspecific heteroantibodies. RF serum titers were determined by the highest serum dilution giving hemagglutination. Each batch was titrated in international units by milliliter according to the international reference preparation of rheumatoid arthritis serum. RF titers were expressed as international units per milliliter and, according to the manufacturer’s instructions, were considered significant when higher than 12 IU/mL.

Statistical analysis

Continuous variables were compared in the 2 groups with the use of Wilcoxon rank-sum test. Qualitative variables were analyzed with a chi-square test or Fisher exact test when expected frequencies were lower than 5. The potential confounding effect of covariables on the relation was assessed by multivariate analysis (Mantel-Haenszel or Fisher exact test for qualitative variables and exact logistic regression for quantitative variables).

Results

Prior to transplantation, anti-HLA IgG Abs were present in 3 of 24 PBHSC transplant recipients (0 of 24 by CDC, 3 of 24 by Flow-PRA) and in 7 of 27 BM transplant recipients (1 of 27 by CDC, 7 of 27 by Flow-PRA). In addition, anti-HLA IgG Abs were found in 3 of 24 PBHSC transplant donors before G-CSF treatment (1 of 24 by CDC, 3 of 24 by Flow-PRA) and in 2 of 27 BM transplant donors (0 of 27 by CDC, 2 of 27 by Flow-PRA). All 5 HLA-immunized donors were women, with 4 of them having a history of pregnancy (3 of 3 in the PBHSC group, 1 of 2 in the BMT group). Anti-HLA Abs were never simultaneously detected in the recipient (before transplantation) and in the donor.

Determination and titration of anti-HLA Abs before and after G-CSF treatment were performed in 19 PBHSC transplant donors. Treatment by G-CSF did not result in the early (within 24 hours after the end of G-CSF treatment) appearance of anti-HLA Ab in any of the 16 anti-HLA–negative (pre–G-CSF) donors. The 3 donors with anti-HLA IgG Abs before G-CSF treatment had similar Ab titers after G-CSF. However, in one of these donors, post–G-CSF evaluation revealed, in addition to a known anti-HLA class II IgG Ab immunization, the presence of an anti-HLA class I IgG Ab.

The use of a PBHSC allogeneic graft was associated with an increased frequency of anti-HLA IgG Abs detected at day 30 (Tables 2 and 3). The increased frequency of anti-HLA IgG Abs did not reach significance when Abs were detected by CDC (5 of 24 in PBHSC transplant recipients versus 1 of 27 in BM transplant recipients, P = .09) (Table 2). However, when Abs were detected by flow cytometry, PBHSCT was significantly associated with an increased frequency of anti-HLA Abs (11 of 24 PBHSC transplant recipients versus 4 of 27 BM transplant recipients, P = .03). Anti-HLA IgG Abs were directed against both HLA class I Ags (10 of 24 transplant recipients after PBHSCT versus 3 of 27 after BMT, P = .02) and/or HLA class II Ags (5 of 24 versus 1 of 27 transplant recipients, P = .09) (Table 3). In 4 patients, all from the PBHSCT group, both IgG class I and IgG class II HLA Abs were detected.

To more accurately measure the influence of the type of HSC graft on the de novo occurrence of anti-HLA Ab after PBHSCT or BMT, we restricted our analysis to anti-HLA Ab–negative recipients (prior to transplantation) having received a graft from an anti-HLA Ab–negative donor. In this group of recipients, the early presence of anti-HLA IgG Ab was detected only after PBHSCT and never after BMT. The association between a PBHSCT graft and the increased occurrence of anti-HLA IgG Abs was confirmed in this group of recipients both by CDC (4 of 23 in PBHSC transplant recipients versus 0 of 26 in BM transplant recipients, P = .04) and by flow cytometry (anti-HLA class I Abs, 7 of 20 after PBHSCT versus 0 of 20 after BMT, P = .008; anti-HLA class II Abs, 1 of 19 versus 0 of 22, P = .46) (Tables 2 and 3). De novo occurrence of both anti-HLA class I and II IgG Abs were observed in one PBHSC transplant recipient.

When the recipient had detectable anti-HLA class I and/or class II IgG Abs prior to transplantation (3 of 24 PBHSC transplant recipients, 7 of 27 BM transplant recipients), such Abs were detected at day 30 after transplantation in 2 of 3 PBHSC transplant recipients versus 3 of 7 BM transplant recipients. On the other hand, the presence in the donor of anti-HLA class I and/or class II IgG Abs before graft harvest (3 of 24 PBHSC donors, 2 of 27 BM donors) was associated with the detection of anti-HLA IgG Abs at

Table 2. Occurrence of serum anti-HLA Abs detected by CDC 30 days after BMT or PBHSCT

<table>
<thead>
<tr>
<th>Ab isotype</th>
<th>Donors</th>
<th>Recipients pretransplantation</th>
<th>Recipients at day +30</th>
<th>Recipients at day +30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PBHSCT (%)</td>
<td>BMT (%)</td>
<td>P</td>
</tr>
<tr>
<td>Anti-HLA IgG Ab</td>
<td>All</td>
<td>5/24 (21)</td>
<td>1/27 (4)</td>
<td>.09</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>4/23 (17)</td>
<td>0/26 .04</td>
<td></td>
</tr>
<tr>
<td>Anti-HLA IgM Ab</td>
<td>All</td>
<td>8/24 (33)</td>
<td>0/27 .001</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Occurrence of serum anti-HLA IgG Ab detected by Flow-PRA 30 days after BMT or PBHSCT

<table>
<thead>
<tr>
<th>Ab isotype</th>
<th>Donors</th>
<th>Recipients pretransplantation</th>
<th>Recipients at day +30</th>
<th>Recipients at day +30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PBHSCT (%)</td>
<td>BMT (%)</td>
<td>P</td>
</tr>
<tr>
<td>Anti-HLA class I IgG</td>
<td>All</td>
<td>10/24 (42)</td>
<td>3/27 (11)</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1/1</td>
<td>1/2</td>
<td>—</td>
</tr>
<tr>
<td>Anti-HLA class II IgG</td>
<td>All</td>
<td>5/24 (21)</td>
<td>1/27 (4) .09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1/2 (50)</td>
<td>1/4 (25)</td>
<td>—</td>
</tr>
</tbody>
</table>
day 30 in 3 of 3 PBHSC transplant recipients and 1 of 2 BM transplant recipients. Finally, when the donor had anti-HLA Abs with identified HLA specificity, similar Ab specificity was found in the recipient when Abs were present at day 30 (data not shown).

PBHSCT was also associated with an increased incidence of anti-HLA IgM Ab after PBHSCT versus after BMT (8 of 24 PBHSC transplant recipients versus 0 of 27 BM transplant recipients, \( P = .001 \)). This finding also persisted after the exclusion of the sole recipient (BMT recipient) who received a graft from a donor with anti-HLA IgM Ab: 8 of 24 PBHSC transplant recipients versus 0 of 26 BM transplant recipients (\( P = .001 \)) (Table 2). No anti-HLA IgM Abs were found in recipients before transplantation.

In addition to pretransplantation anti-HLA immune status, both BMT and PBHSCT donor/recipient groups were compared for a number of parameters that might have induced the occurrence of anti-HLA Ab 30 days after transplantation. As detailed in Table 1, pretransplantation parameters such as age, sex and sex mismatch, previous pregnancy, diagnosis, and conditioning regimen did not differ significantly in the 2 groups. Three posttransplantation parameters could significantly influence the occurrence of anti-HLA Ab: IVIG treatment, RBC-C transfusions, and PC transfusions (Table 4). IVIG treatment and RBC-C transfusion did not significantly differ in the PBHSCT and BMT groups. In contrast, the median number of PC transfusion episodes was significantly lower in the PBHSCT group than in the BMT group (3 platelet-transfusion episodes [range, 1-21] in PBHSCT group vs 6 platelet-transfusion episodes [range, 3-33] in the BMT group; \( P = .02 \)). After adjustment for each of the potential confounding variables in bivariate analyses, the use of a PBHSC graft remained significantly associated with an increased frequency of de novo anti-HLA IgG (CDC and Flow-PRA, class I) as well as IgM Abs.

The occurrence of anti-HLA Abs after hematopoietic transplantation has been reported\(^1\) and could result from the passive transfer of anti-HLA Ab (prior presence in the recipient; plasma and/or IVIG administration) or from donor transfer of a known or unknown (ie, undetected) anti-HLA immunity. To further explore this last issue, we examined, within the group of PBHSCT transplant recipients with no detectable pretransplant recipient or donor anti-HLA immunization, whether the presence of anti-HLA Abs indeed occurred more frequently when there was a female donor with a known history of pregnancy. No evidence for significant association was found. However, there was indeed a trend for a higher frequency of de novo immunization when the donor was a female with a history of pregnancy versus a male donor or a female donor with no known history of pregnancy (CDC, 3 of 7 versus 1 of 16, \( P = .07 \); Flow-PRA anti-HLA class I, 4 of 7 versus 3 of 13, \( P = .17 \)) (Table 5). Furthermore, 2 recipients with de novo presence of anti-HLA Ab at day 30 received a graft from a male donor with no prior transfusion history, thus suggesting that de novo posttransplantation immunization also contributed—at least in part—to the presence of anti-HLA Ab at day 30.

In view of the significant association between the use of PBHSC graft and the occurrence of serum anti-HLA IgM Ab after transplantation, we chose to evaluate the possible influence of the type of HSC graft on the appearance of nonalloreactive IgM Ab such as RF. At 30 days after transplantation, the presence of RF was detected in only 1 of 18 PBHSC transplant recipients and in 1 of 20 BM transplant recipients. RF titers were high in the PBHSC transplant recipient (latex, 91.3 IU/mL; Waaler-Rose, 512 IU/mL) but borderline positive in the BM transplant recipient (latex, 13 IU/mL; Waaler-Rose, below 8 IU/mL).

Serum samples collected between 1 and 2 years after transplantation were available for a limited number of recipients (\( n = 18 \)). Flow-PRA analysis revealed the presence of anti-HLA Abs in 1 of 9 PBHSC graft recipients and in 1 of 9 BM graft recipients.

**Discussion**

In our study, the use of an allogeneic PBHSC graft was associated with an increased frequency of anti-HLA Abs 30 days after transplantation as compared—in a randomized setting—with a BM graft. This result persisted after adjustment for each potential confounding factor, such as age, sex mismatch, transfusion practices, and IVIG administration, known to possibly affect immune reconstitution after transplantation. Importantly, the PBHSCT-associated increase in detected anti-HLA Abs was observed despite a reduction in the median number of platelet transfusion episodes per patient in PBHSC transplant versus BM transplant recipients.

De novo detection of circulating anti-HLA Abs tended to occur with a higher frequency in female donors with a history of pregnancy versus male donors or female donors with no known history of pregnancy. This finding suggests that after PBHSCT, previously unknown immunization may have been boosted by the use of a G-CSF–mobilized graft and posttransplantation RBC-c or PC transfusion. On the other hand, among the donors of the PBHSCT transplants to the recipients with first detected anti-HLA Abs, 2 out of 7 were male donors with no history of transfusion or intravenous drug use. Such a finding suggests the possible occurrence of primary anti-HLA alloimmunization after PBHSCT despite the reduced PC transfusion requirements and the systematic use of leuko-reduced blood products. However, in both cases, the limited number of informative recipients or donors prevents any definitive conclusion.

Because PBHSCT was associated with accelerated platelet reconstitution, any deleterious effects on platelet transfusion requirements related to the increased presence of anti-HLA Abs might not be discernible. Furthermore, the accelerated hematopoietic reconstitution associated with PBHSCT might have prevented any measurable anti-HLA Ab–related decrease in transfusion efficacy. Finally,

| Table 4. IVIG administration and transfusion episodes after transplantation |
|-----------------------------|------------------------------|---------------------|---------|
| \( \frac{\text{PBHSC transplant recipients, } N = 24}{\text{BM transplant recipients, } N = 27} \) | \( P \) |
| **Patients receiving IVIG** | | | |
| Yes (\( N = 24 \)) | 10 (42%) | 14 (52%) | .47 |
| No (\( N = 27 \)) | 14 (58%) | 13 (48%) | .47 |
| **Transfusions** | | | |
| RBC episodes, median (range) | 3 (0–8) | 3 (0–7) | .29 |
| PC episodes, median (range) | 3 (1–21) | 6 (3–33) | .02 |

**Table 5. Relation between de novo anti-HLA IgG Ab occurrence 30 days after PBHSCT and prior donor pregnancy**

<table>
<thead>
<tr>
<th>Anti-HLA IgG*</th>
<th>Parous females</th>
<th>Nonparous females or males</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HLA class I IgG†</td>
<td>3/7</td>
<td>1/16</td>
<td>.07</td>
</tr>
<tr>
<td>Anti-HLA class II IgG†</td>
<td>4/7</td>
<td>3/13</td>
<td>.17</td>
</tr>
<tr>
<td>Anti-HLA class III IgG†</td>
<td>1/5</td>
<td>0/14</td>
<td>.26</td>
</tr>
</tbody>
</table>

*Detected by CDC.†Detected by Flow-PRA.
all patients included in our study received methotrexate at days 1, 3, and 6 after transplantation. Methotrexate is cytotoxic for B lymphocytes and might have contributed to the delay of the appearance of anti-HLA Abs until after platelet reconstitution.

As mentioned earlier, we have observed that the use of a PBHSC graft was also associated with increased anti-A and/or anti-B Ab titers early after transplantation. Such increased Ab titers probably account for the occurrence of several acute hemolysis episodes after allogeneic PBHSCST in the setting of a “minor” ABO mismatch and the absence of methotrexate in the GVHD prophylaxis regimen. These hemolysis episodes occurred most often between day 8 and day 14 after PBHSCST and were associated with the production of Abs directed at ABO Ags present on recipient RBCs. In this respect, we have shown that PBHSC recipients indeed exhibited significantly increased anti-A and/or anti-B Ab titers at day 30 following PBST and particularly in the setting of a minor ABO mismatch.

While it seems most likely that the increased anti-HLA antibody production is donor derived, formal proof is lacking. The number of B cells circulating at day 30 is too low, to allow for B-cell chimerism determination. Furthermore, isolating the anti-HLA Ab among the serum Ig for subsequent GM typing is a difficult task and would have required larger serum samples.

Quantitative and qualitative differences between PBHSC and BM grafts could contribute to such an increase in early anti-HLA Ab or anti-AB Ab responses after PBHSCST. The higher number of B cells (especially those expressing CD45RO, CD25, or CD23 activation markers), T cells, and monocytes present in the PBHSC harvest, as compared with BM graft, could be associated with an enhanced Ab production early after PBHSCST. Furthermore, we have also found that, early after transplantation, peripheral blood counts of most lymphocyte subsets, including CD4 T cells and B cells, were higher in PBHSC graft recipients.

We have previously demonstrated that G-CSF mobilization enhances the expression of CD45RO by CD19+ cells contained in the graft. Acquisition of CD45RO expression by cells has been associated with in vivo or in vitro B-cell transition from mature B-cell stage to early preplasma cells. Therefore, G-CSF mobilization could have a “priming” effect on B cells and render these cells more susceptible to Ag-induced activation than BM-associated B cells. The observation in vitro that G-CSF enhanced Ig generation, rather than B-cell proliferation, further strengthens this hypothesis.

G-CSF–induced Tpl2 cytokine profile of the T cells present in the graft could possibly also contribute to enhance post-PBHSCT Ab responses. We have determined that the frequency of interferon-γ (IFN-γ)–producing T cells as well as the capacity to produce IFN-γ at the single-cell level is indeed reduced in a PBHSC graft versus a BM graft. Furthermore, reduced tumor necrosis factor-α production and increased interleukin 10 (IL-10) production have been attributed to G-CSF exposure. Finally, recent studies have determined that G-CSF–mobilized PBHSC grafts contained a higher number of type 2 dendritic cells (DC2). Such G-CSF–induced DC2 do not produce IL-12 and are associated with high frequencies of IL-4– and IL-10–producing CD4+ cells not expressing the IL-12 receptor beta 2 chain. After infusion to the recipient, such DC2 could induce type 2 immune reactivity, including enhanced Ab responses.

Overall circulating immunoglobulin levels were found to be similar 80 days after PBHSCST versus after BMT in a study recently reported by Storek et al. We were unable to detect an increased occurrence of circulating RF early after PBHSCST. To determine whether or not increased Ab response early after PBHSCST is preferentially directed against allogeneic Ags will require additional studies.

An increased incidence of chronic GVHD after PBHSCST when compared with BMT has been observed in our study and confirmed in a recent meta-analysis. Since chronic GVHD is characterized by the frequent occurrence of Ab-mediated autoimmune-like syndromes, it is tempting to speculate that such a higher incidence of chronic GVHD may result, at least in part, from the higher level of B-cell activation and/or higher number of circulating B cells after PBHSCST with consequently increased Ab responses.

In conclusion, our study strongly suggests that G-CSF–mobilized PBHSCST results in an increased incidence of anti-HLA immunization and further confirms that the use of different hematopoietic stem cell sources is associated with distinct immune-reconstitution patterns. Further dissection of such differences should contribute to enhancing the quality of clinical practice in patients requiring allogeneic HSC transplantation.

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