Increased presence of anti-HLA antibodies early after allogeneic G-CSF-mobilized blood hematopoietic stem cell transplantation compared to bone marrow transplantation

**Short title:** Anti-HLA Ab after blood stem cell transplantation

**Scientific Section Heading:** Transplantation

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Abstract

We have recently shown that the use of allogeneic G-CSF mobilized peripheral blood hematopoietic stem cell transplantation (PBHSCT) compared with bone marrow transplantation (BMT) is associated with increased titers of antibodies (Ab) directed against red blood cell ABO antigens (Ag). To further evaluate the influence of a G-CSF mobilized PBHSC graft on allo-immune Ab responses, we examined the frequency of anti-HLA Ab after transplantation in the setting of the same randomized study comparing PBHSCT vs BMT in adults. Anti-HLA Ab presence was determined by complement-dependent lymphocytotoxicity (CDC) and flow-cytometry in the recipient before and 30 days after transplantation as well as in the donor. The use of PBHSCT was significantly associated with an increased detection of anti-HLA IgG Ab early after transplantation as evidenced by flow-cytometry (11/24 vs 4/27, p=0.03) and, less so, by CDC (5/24 vs 1/27, p=0.09). Such a 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 while repeatedly detected after PBHSCT (flow cytometry: 6/18 vs 0/17, p=0.02; CDC: 4/23 vs 0/26, p=0.04). Importantly, the PBHSCT-associated increase in anti-HLA Ab detection was observed despite a reduction in the median number of platelet transfusion episodes/patient in PBHSCT vs BMT recipients (3[1-21] vs 6 [3-33], p=0.02). In conclusion, our study strongly suggests that G-CSF mobilized PBHSCT results in an increased incidence of circulating anti-HLA-Ab and further confirms that the use of such a graft alters allo-immune Ab responses.

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Introduction

Use of peripheral blood stem cells after 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 impacts significantly on the number and function of immune cells present in the graft as well as on post-transplantation immune reconstitution when compared to a bone marrow (BM) graft (8, 12-17). In particular, we have recently shown that the use of allogeneic G-CSF mobilized peripheral blood HSC transplantation (PBHSCT) can alter an allo-immune antibody (Ab) response as evidenced by increased titers of Ab directed against red blood cell (RBC) ABO antigen (Ag) after PBHSCT when compared to BM transplantation (BMT) (16). Such an altered immuno-hematological 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 anti-HLA Ab occurrence after transplantation, we have taken advantage of the same multicenter randomized phase III clinical trial comparing allogeneic BMT to allogeneic PBHSCT conducted by the Société Française de Greffe de Moelle (7) to prospectively compare the frequency of anti-HLA Ab detection after allogeneic PBHSCT vs BMT.
Material and method

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 comparing allogeneic PBHSCT vs 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 in the respect of the Helsinki accords for human subject research. All procedures were performed after donors and recipients gave written informed consent. In June 1998, a prospective immuno-biological evaluation was initiated for patients subsequently entered in the study. This evaluation included assessment of the donor [before G-CSF administration and less than 24 hours after the last G-CSF infusion (PBHSCT) and at time of HSC graft harvest (BMT)] and assessment of the recipient before and 30 days after transplantation. From June 1998 to June 1999, 51 of 73 consecutive randomized patients (67%) (PBHSCT, n=24; BMT, n=27) were included in the present study. Twenty patients were not included because of missing (non-harvested 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 (7). 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/day of sub-cutaneous G-CSF (Filgrastim; Rhône-Poulec Rhorer Co, Montrouge, France) for 5 days. On the 5th day (day -1 of transplantation), the first HSC harvest was performed by apheresis. If CD34+ cell counts in the HSC bag were less than 4x10^6/kg of recipient body weight, a second harvest was performed on the 6th day. G-CSF was administered on the 6th day if a third harvest was required at day +1. GvHD prophylaxis comprised cyclosporin A (initiated at day -1) and methotrexate (15mg/m^2 on day +1; 10 mg/m^2 on days +3 and +6). Cyclosporin A was started intravenously on day -1 at the dose of 2 to 3 mg/kg/day, and switched to oral formulation as soon as oral intake was satisfactory. The dosage was adapted to whole blood or plasma level and renal function according to each center’s practice. No recipient received G-CSF during the HLA immunological study period.
Table 1: Donors and recipients 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><strong>Age</strong> (median [extremes])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donors</td>
<td>33 (22;51)</td>
<td>33 (20;63)</td>
<td>0.70</td>
</tr>
<tr>
<td>Recipients</td>
<td>35 (1.6;51)</td>
<td>34 (16;50)</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Sex mismatch</strong> (donorrecipient)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FemaleFemale [parous donor]</td>
<td>5 (21%) [2]</td>
<td>3 (11%) [2]</td>
<td></td>
</tr>
<tr>
<td>MaleFemale</td>
<td>4 (17%)</td>
<td>10 (37%)</td>
<td></td>
</tr>
<tr>
<td>MaleMale</td>
<td>9 (38%)</td>
<td>9 (33%)</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Donor</strong></td>
<td></td>
<td></td>
<td></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>0.33</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></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>0.70</td>
</tr>
<tr>
<td><strong>Conditioning Regimen</strong></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>0.18</td>
</tr>
</tbody>
</table>

BMT: bone marrow haematopoietic stem cell transplantation; PBHSCT: peripheral blood haematopoietic stem cell transplantation
ALL: acute lymphoid leukaemia, AML: acute myeloid leukaemia, CML: chronic myeloid leukaemia
TBI: total body irradiation (median: 12 Gy [range: 11-13.5]), EDX: cyclophosphamide (120 mg/kg), BUS: busulfan (16mg/kg per os), VP16: vepeside (20 mg/kg), TAME: TBI + aracytine (12 g/m2) + melphalan (140 mg/m2) + etoposide (60mg/kg)
**Platelet concentrate (PC) and RBC concentrate (RBC-C) transfusion**

HSC donor and recipient ABO-compatible RBC-C were transfused when the haemoglobin level was below 8 g/dl according to transfusion practices previously described (24). Single donor PC were administered to treat or prevent haemorrhage when blood platelet counts were below 20x10⁹/L. All RBC-C and PC were leukoreduced by pre-storage filtration. The number and time of administration of PC and RBC-C were recorded for all recipients. Similarly, administration of intravenous polyvalent immunoglobulins (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 PBHSC 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 post-transplant (n=51). In addition, whenever possible, “long term” serum samples were obtained from BM (n=9) or PBSCH (n=9) recipients between one to two years after transplantation. Blood samples were shipped by overnight mail to the Etablissement Français du Sang Bourgogne-Franche-Comté 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) (25) and by flow-cytometry (Flow-PRA®) (26-28). This latter method, recently developed, detects both anti-HLA class I and anti-HLA class II IgG Ab with a higher sensitivity when compared to CDC (28).

The CDC assays were performed as previously described (25) 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 percent cytotoxicity in one or more cell populations or at least 40 percent cytotoxicity in two or more cell populations of the panel. In such a case, positivity was confirmed on a second panel of 30 different HLA-typed donors and tested in the presence or absence of dithiothreitol (DTT). In the case of anti-HLA IgG Ab, positivity persisted despite treatment with DTT while the presence of IgM Ab 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 a method that allows a clear distinction between anti-HLA class I or II IgG Ab, CDC testing against purified HLA-typed B cells after absorption on platelets was not performed.

Flow-PRA® (One-Lambda, Canoga Park, CA) assays were performed according to the supplier’s recommendations. Briefly, 20 µl of test serum were incubated for 30
min with 5 µl of class-I and 5 µl of class-II beads at 22°C. The beads were then washed twice with buffer and incubated, for 30 min, with 100 µl of FITC-conjugated goat anti-human IgG at 22°C in the dark. After two additional washings, the beads were fixed in 1% paraformaldehyde buffer. In each assay, negative and positive controls were, respectively: (a) pooled AB sera tested by CDC and flow-cytometry on three different cell populations, and (b) a pool of polyspecific anti-HLA Ab sera. Analysis was done with a FaCS-SCAN (Becton Dickinson) on a 1024-channel linear scale. In the assays performed to determine a standardized cut-off 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 (28).

Detection of Rheumatoid Factors

Quantitative determination of serum Rheumatoid Factor (RF) was performed by nephelometry and haemagglutination. Nephelometry measured the endpoint aggregation of small latex particles coated with human IgG (N Latex RF kit and BNII nephelometer, Dabe Behring Marburg GmbH, Marburg, Germany). Microplate haemagglutination technique (Laboratoires Fumouze division diagnostics, Levallois-Peret, France), based on the principle of the Waaler-Rose reaction (29), used sheep erythrocytes sensitised by the IgG fraction of rabbit anti-sheep erythrocyte serum. The latter technique discriminates RF and non specific hetero-Ab. Rheumatoid factor serum titers were determined by the highest serum dilution giving haemagglutination. As each batch was titrated in international units by milliliter with respect to the
international reference preparation of Rheumatoid Arthritis serum (30), RF titers were expressed as international units by milliliter and considered significant when higher than 12 IU/ml according to the manufacturer’s instructions.

**Statistical analysis**

Continuous variables were compared between the two groups using Wilcoxon rank-sum test. Qualitative variables were analyzed with a chi-square test, or exact test when expected frequencies were lower than 5. The potential confounding effect of covariables on the relation between source of HSC and first detected anti-HLA Ab (de novo anti-HLA Ab) in the recipient at day +30 were studied one by one by bivariate analysis (Mantel-Haenszel or exact methods for qualitative variables and exact logistic regression for quantitative variables).
Results

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

Determination and titration of anti-HLA Ab before and after G-CSF treatment were performed in 19 PBHSCT 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 Ab before G-CSF treatment had similar Ab titers after G-CSF. However, in one of these donors, post-G-CSF evaluation revealed the presence, in addition to a known anti-HLA class II IgG Ab immunization, an anti-HLA class I IgG Ab.

The use of a PBHSC allogeneic graft was associated with an increased frequency of anti-HLA IgG Ab detected at day 30 (Table 2 and 3). This increased frequency of anti-HLA IgG Ab did not reach significance when detected by CDC (5/24 in PBHSCT recipients vs 1/27 in BMT recipients [p=0.09])(Table 2). However, when detected by flow-cytometry, PBHSCT was significantly associated with an increased frequency of anti-HLA Ab (11/24 vs 4/27 in BMT recipients [p=0.03]). Anti-HLA IgG Ab were
directed both against HLA class I Ag (10/24 after PBHSCT vs 3/27 after BMT, 
p=0.02) and/or HLA class II Ag (5/24 vs 1/27, p=0.09) (Table 3). In 4 patients, all 
from the PBHSCT group, both IgG class I and II HLA Ab were detected.

<table>
<thead>
<tr>
<th>Anti-HLA Ab isotype</th>
<th>Presence of anti-HLA Ab</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor</td>
<td>Pre-transplant</td>
<td>Recipient at day +30</td>
<td>PBHSCT (%)</td>
<td>BMT (%)</td>
</tr>
<tr>
<td>IgG</td>
<td>All</td>
<td>All</td>
<td>5/24 (21%)</td>
<td>1/27 (4%)</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4/23 (17%)</td>
<td>0/26</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/1</td>
<td>-</td>
<td>-</td>
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<td></td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Occurrence of serum anti-HLA antibodies detected by complement-dependent cytotoxicity assay 30 days after BMT or PBHSCT
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 increased occurrence of anti-HLA IgG Ab was confirmed in this group of recipients both by CDC (4/23 in PBHSCT recipients vs 0/26 in BMT recipients [p=0.04]) and by flow-cytometry (anti-HLA class I Ab:7/20 after PBHSCT vs 0/20 after BMT, p=0.008; and/or anti-HLA class II Ab:1/19 vs 0/22, 0.5.
p=0.46)(Table 2 and 3). De novo occurrence of both anti-HLA class I and II IgG Ab was observed in one PBHSCT recipient.

When the recipient had detectable anti-HLA class I and/or class II IgG Ab prior to transplantation (3/24 PBHSCT recipients, 7/27 BMT recipients), such Abs were detected at day 30 after transplantation in 2/3 PBHSCT recipient vs 3/7 BMT recipients. On the other hand, the presence in the donor of anti-HLA class I and/or class II IgG Ab before graft harvest (3/24 PBHSC donors, 2/27 BM donors) was associated with the detection of anti-HLA IgG Ab at day 30 in 3/3 PBHSCT recipients and 1/2 BMT recipients. Lastly, when the donor had anti-HLA Ab with identified HLA specificity, similar Ab specificity was found in the recipient when Ab were present at day 30 (data not shown).

PBHSCT was also associated with an increased incidence of anti-HLA IgM Ab after PBHSCT vs after BMT (8/24 PBHSCT recipients vs 0/27 BMT recipients, p=0.001). 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: 8/24 in PBHSCT recipients vs 0/26 BMT recipients (p=0.001)(Table 2). No anti-HLA IgM Ab were found in recipients before transplantation.

In addition to pre-transplant 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, pre-transplantation parameters such as age, sex and sex-mismatch, previous pregnancy, diagnosis and conditioning regimen were parameters that did not differ significantly between both groups. Three post-transplant parameters could significantly
influence on the occurrence of anti-HLA Ab: IVIG treatment as well as RBC-C and PC transfusions (Table 4). IVIG treatment and RBC-C transfusion did not significantly differ between 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 [1-21] vs 6 [3-33], p=0.02). 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 Ab.

Table 4: IVIG administration and transfusion episodes after transplantation

<table>
<thead>
<tr>
<th></th>
<th>PBHSCT recipients</th>
<th>BMT recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=24</td>
<td>N=27</td>
</tr>
<tr>
<td>Patient receiving IVIG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (N=24)</td>
<td>10 (42%)</td>
<td>14 (52%)</td>
</tr>
<tr>
<td>No (N=27)</td>
<td>14 (58%)</td>
<td>13 (48%)</td>
</tr>
<tr>
<td>Transfusions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBCs episodes median [extremes]</td>
<td>3 (0;8)</td>
<td>3 (0;7)</td>
</tr>
<tr>
<td>PC episodes median [extremes]</td>
<td>3 (1;21)</td>
<td>6 (3;33)</td>
</tr>
</tbody>
</table>

RBC: red blood cells concentrates; PC: platelets concentrates; IVIG: intravenous immunoglobulins

The occurrence of anti-HLA Ab after hematopoietic transplantation has been reported (31) and could result from the passive transfer of anti-HLA Ab (prior presence in the recipient; plasma and/or IVIG administration), 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 recipients with no detectable pre-transplant recipient or donor anti-HLA immunization, whether the presence of anti-HLA Ab occurred indeed more frequently in the presence of a female donor with a known history of pregnancy. No significant
association was evidenced. 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 vs a male or female donor with no known history of pregnancy (CDC: 3/7 vs 1/16, p=0.07; Flow-PRA anti-HLA class I: 4/7 vs 3/13, p=0.17)(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 post-transplantation, transfusion induced immunization also contributed -at least in part- to the presence of anti-HLA Ab at day 30.

**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 Ab</th>
<th>Parous Females</th>
<th>Non-parous female or Male</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HLA IgG*</td>
<td>3/7</td>
<td>1/16</td>
<td>0.07</td>
</tr>
<tr>
<td>Anti-HLA class-I IgG**</td>
<td>4/7</td>
<td>3/13</td>
<td>0.17</td>
</tr>
<tr>
<td>Anti-HLA class-II IgG**</td>
<td>1/5</td>
<td>0/14</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*detected by complement-dependent cytotoxicity assay
**detected by Flow-PRA

In view of the significant association between the use of PBHSCT 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 non-alloreactive IgM Ab such as RF. Thirty days after transplantation, the presence of RF was detected only in 1/18 PBHSCT recipient and in 1/20 in the BMT recipient. Rhumatoid Factor titers were high in the PBHSCT recipient (latex: 91.3 IU/ml; Waaler-Rose: 512 IU/ml) while borderline positive in the BMT recipient (latex: 13 IU/ml; Waaler-Rose: <8 IU/ml).
Serum samples collected between one to two years after transplantation were available for a limited number of recipients (n=18). Flow-PRA analysis revealed the presence of anti-HLA Ab in 1/9 PBSHC graft recipients and in 1/9 BM graft recipients.
Discussion

In our study, the use of an allogeneic PBHSC graft was associated with an increased frequency of anti-HLA Ab 30 days after transplantation when compared - in a randomized setting - to 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 anti-HLA detected Ab was observed despite a reduction in the median number of platelet transfusion episodes/patient in PBHSCT vs BMT recipients.

De novo detection of circulating anti-HLA Ab tended to be observed with a higher frequency in female donor with a history of pregnancy vs male 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 post-transplant RBC or PC transfusion. On the other hand, among the donors of the PBHSCT recipients with first detected anti-HLA Ab, 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 platelet transfusion requirements and the systematic use of leukoreduced 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
Ab might not be discernible. Furthermore, the accelerated haematopoietic reconstitution associated with PBHSCT might have prevented any measurable anti-HLA Ab-related decrease in transfusion efficacy. Lastly, all patients included in our study received methotrexate at d+1, +3 and +6 after transplantation. Methotrexate is cytotoxic for B lymphocytes (32) and might have contributed to delay the appearance of anti-HLA Ab 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 B Ab titers early after transplantation (16). Such increased Ab titers probably account for the occurrence of several acute hemolysis episodes after allogeneic PBHSCT in the setting of a “minor” ABO mismatch and the absence of methotrexate in the GvHD prophylaxis regimen (18-23). These hemolysis episodes occurred most often between day 8 and day 14 after PBHSCT and were associated with the production of Ab directed at ABO Ag present on recipient RBC. 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 PBSCT and particularly in the setting of a “minor” ABO mismatch (16).

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 are 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 (13, 14) could contribute such an increase in early anti-HLA or - AB Ab responses after PBHSCT. The higher number of B cells (and 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 PBHSCT. Furthermore, we (15) and others (17) 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 (14). 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 pre-plasma cells (33,34). Therefore, G-CSF mobilization could have a « priming » effect on B cells and rend these cells more susceptible to Ag-induced activation than BM-associated B cells. The observation in vitro that G-CSF enhanced immunoglobulin generation, rather than B cell proliferation (35), further strengthens this hypothesis.

G-CSF induced TH2 cytokine profile of the T cells present in the graft could also possibly contribute to enhance post-PBHSCT Ab responses (36,37). We have determined that the frequency of IFN-gamma-producing T cells as well as the capacity of producing IFN-gamma at single cell level is indeed reduced in a PBHSC graft vs a BM graft (13). Furthermore, reduced TNF-alpha production (38) and increased IL-10 production (39) have been attributed to G-CSF exposure. Lastly, recent studies have determined that G-CSF mobilized PBSC grafts contained a
higher number of type 2 dendritic cells (DC2) (40,41). Such G-CSF induced DC2 cells 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 (41). After infusion to the recipient, such DC2 cells could induce type 2 immune reactivity, including enhanced antibody responses.

Overall circulating Ig levels were found to be similar 80 days after PBHSCT vs BMT in a study recently reported by Storek et al (17). We were unable to detect an increased occurrence of circulating RF early after PBHSCT. To determine whether or not increased Ab response early after PBHSCT is preferentially directed against allogeneic Ag will require additional studies.

An increased incidence of chronic GvHD after PBHSCT when compared to BMT has been observed in our study (7) and confirmed in a recent meta-analysis (11). Since chronic GvHD is characterized by the frequent occurrence of Ab-mediated autoimmune-like syndromes (42), 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 PBHSCT with consequently increased Ab responses.

In conclusion, our study strongly suggests that G-CSF mobilized PBHSCT 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 enhance the quality of clinical practice in patients requiring allogeneic HSC transplantation.
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Increased presence of anti-HLA antibodies early after allogeneic 
G-CSF-mobilized blood hematopoietic stem cell transplantation 
compared to bone marrow transplantation

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