Reconstitution of Antibody Response After Allogeneic Bone Marrow Transplantation: Effect of Lymphocyte Depletion by Countercflow Centrifugal Elutriation on the Expression of Hemagglutinins

By B.M.A.M. Bär, G.W. Santos, and A.D. Donnenberg

The generation of ABO hemagglutinins was used as a model to assess the tempo of reconstitution of antibody responses in recipients of elutriated (CCE) and nonelutriated (nonCCE) HLA matched bone marrow allografts. The study included 29 CCE recipients (10 major, 6 minor, and 1 major/minor ABO-mismatched, and 12 ABO-matched) and 40 nonCCE recipients (14 major, 12 minor, 2 major/minor, and 12 matched). Plasma volume in the graft and in blood product transfusions was uncorrelated with changes in hemagglutinin titers and therefore was excluded as a significant source of antibody. Removal of graft lymphocytes by CCE did not result in prolongation of host-derived hemagglutinins in recipients of major ABO-mismatched grafts. However, CCE resulted in a complete abrogation of the adoptive transfer of donor-derived antibody as detected in recipients of minor ABO-mismatched grafts.

Despite the absence of adoptively transferred donor immunity in recipients of CCE grafts, they had hemagglutinin levels comparable with those of recipients of nonCCE grafts by 6 months after transplantation. This demonstrates that recipients of elutriated marrow were competent to mount de novo responses at that time. The strong correlation between donor pretransplant hemagglutinin titer and recipient titer 1 year after bone marrow transplantation in recipients of nonCCE grafts suggests that even late after transplant, antibody remains the product of adoptively transferred memory cells in recipients of grafts containing large numbers of mature lymphocytes.

COUNTERFLOW centrifugal elutriation (CCE) has emerged as an efficient and safe methodology for the removal of lymphocytes from bone marrow allografts. Lymphocyte depletion by this method effectively prevents acute graft-versus-host disease (AGVHD) in a lymphocyte dose-dependent fashion in patients at high risk for this complication of bone marrow transplantation (BMT). Beyond simple depletion, CCE has proven to be a flexible technique capable of subfractionating lymphocytes into functionally distinct subpopulations. This may ultimately open the way for immunologic graft engineering. To realize this potential, it is important to closely document immune reconstitution in recipients of elutriated marrow and assess the impact of this procedure by comparison with patients transplanted with conventional allografts. By evaluating the expression of hemagglutinins we have implemented a simple, biologically meaningful method for the prospective evaluation of the development of T- and B-lymphocyte dependent immunity. Further, the availability of ABO-mismatched donor/recipient pairs has enabled us to ascribe the origin of particular antibody specificities uniquely to donor or recipient, and track the course of their disappearance or emergence as a function of graft type (elutriated or nonelutriated) and time after BMT. Apart from the practical need to evaluate the effects of lymphocyte depletion on immune reconstitution, this methodology has also permitted us to dissect the relative contributions of the various components that can potentially influence the reconstitution of antibody responses. These include passively transferred antibody, residual recipient responses, donor immune memory cells adoptively transferred in the graft, and lymphocytes of donor origin that have differentiated within the host environment.

MATERIALS AND METHODS

Mismatch definitions. For the purpose of analysis, donor and recipient ABO disparities were divided into two groups depending on the direction of potential immune recognition. Major ABO incompatibility was defined as a mismatch in which the recipient had the potential to express hemagglutinins directed against donor ABO antigens (ie, recipient antidonor antibody). In minor ABO mismatch, the donor had the potential to express hemagglutinins directed against ABO antigens on recipient erythrocytes (donor antirecipient antibody). Donor and recipient ABO blood group typing and expected antibody specificities are given for the different combinations of ABO-mismatched BMT (Table 1).

Patient population. Between December 1985 and January 1989, 64 patients underwent allogeneic BMT with an elutriated (CCE), HLA-matched graft. In 17 of these patients there was an ABO incompatibility between recipient and donor, 10 cases with major, 6 cases with minor, and 1 case with both major and minor incompatibility (Table 1). The hemagglutinin titers of these 17 patients were compared with those of 28 recipients of HLA-matched, ABO-mismatched, nonelutriated grafts between 1983 and 1989 (Table 1). These 28 patients were participants in an immune reconstitution study, and frozen (−70°C) serial serum samples were therefore available. All 17 recipients of elutriated bone marrow received cyclosporin A (CsA) to prevent graft rejection and graft-versus-host disease (GVHD). Nineteen of the patients with nonelutriated grafts received CsA and nine patients were treated with cyclophosphamide (Cy) for posttransplant GVHD prophylaxis. The median ages in these patient groups were 36, 21, and 22 years, respectively. Patients were transplanted for chronic myelogenous leukemia (CML; eight CCE, seven nonCCE); acute myelogenous leukemia (AML; three CCE, eight nonCCE); acute lymphocytic leukemia (ALL; two CCE, nine nonCCE); non-Hodgkin’s lymphoma (NHL; three CCE, one nonCCE); Hodgkin’s disease (HD; one CCE); and aplastic anemia.
HEMAGGLUTININS IN LYMPHOCYTE-DEPLETED BMT

1411

notypic analysis of represented a high-risk population participating in phase I trials. Patients included in nonCCE grafts plus CsA were also studied (Table 1).

The pretransplant preparative regimen was determined by the patient's admission diagnosis. Patients with AML were treated with busulfan patient's admission diagnosis. Patients with AML were treated with busulfan mg/kg orally for 4 days followed by Cy 50 mg/kg/d for 4 days. Patients with AA were treated with Cy 120 mg/kg plus total body irradiation (TBI) or 200 mg/kg with or without TBI (12 Gy).

The conditioning regimen for ALL, CML, HD, and NHL consisted of Cy 50 mg/kg/d for 4 days followed by TBI (12 Gy over 4 days). An additional 12 recipients of ABO-matched CCE grafts (with CsA chemophrophylaxis) and 12 patients who received ABO matched, nonCCE grafts plus CsA were also studied (Table 1).

For recipients of ABO incompatible transplants, samples were obtained before BMT and 2 weeks, 4 weeks, 3 months, 6 months, and 1 year after transplant. For recipients of ABO-matched grafts, recipient serum samples were examined before BMT, at two intervals early after BMT (2 weeks and 4 weeks), and at one interval late after BMT (1 year in 22 of 24 cases). Sera were stored at -70°C before assay; all sera from a given patient were tested simultaneously. Donor sera obtained before marrow harvest were also assayed at the same time. A total of 362 patient and donor sera were assayed.

Bone marrow processing. All elutriated grafts were quantitatively depleted of erythrocytes (99.94% reduction) and contained no plasma. A predetermined number of lymphocytes was intentionally added to the elutriated grafts. Twenty-two patients included in this study received x 10^6 morphologic lymphocytes/kg ideal body weight, and seven received 0.5 x 10^6 lymphocytes/kg. Immunophenotypic analysis of 11 grafts formulated at 1 x 10^6 lymphocytes/kg indicated a 97% reduction in T cells (CD3+) and a 96% reduction in B cells (CD19+, HLA DR+) compared with the harvested marrow. Nonelutriated grafts were prepared according to the ABO status of the donor and recipient.

To prevent massive hemolysis in ABO-major mismatch, more than 95% of the erythrocytes were removed from the graft by apheresis, after which the bone marrow buffy coat was suspended in 50 to 100 mL of donor plasma. For minor ABO incompatibility, erythrocytes were not depleted, but approximately 90% of the plasma volume was removed from the graft and replaced with saline. Nonelutriated ABO-compatible grafts contained approximately 3 x 10^12 erythrocytes and 600 to 700 mL plasma (mean harvest volume 1,100 mL ± 300).

Table 1. Definition of Expected Antibody Specificity by Patient and Donor Blood Groups

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Expected Antibody Sources and Specificity</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major ABO incompatible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>O</td>
<td>NA*</td>
</tr>
<tr>
<td>B</td>
<td>O</td>
<td>NA</td>
</tr>
<tr>
<td>AB</td>
<td>A</td>
<td>NA</td>
</tr>
<tr>
<td>AB</td>
<td>B</td>
<td>NA</td>
</tr>
<tr>
<td>Major + minor ABO incompatible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>Anti-B</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>Anti-A</td>
</tr>
<tr>
<td>Minor ABO incompatible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>A</td>
<td>Anti-A</td>
</tr>
<tr>
<td>O</td>
<td>B</td>
<td>Anti-B</td>
</tr>
<tr>
<td>O</td>
<td>AB</td>
<td>Anti A, Anti-B</td>
</tr>
<tr>
<td>A</td>
<td>AB</td>
<td>Anti-B</td>
</tr>
<tr>
<td>B</td>
<td>AB</td>
<td>Anti-A</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABO Matched</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>NA</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>NA</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
<td>NA</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NA, not applicable. Antibodies of these specificities are not expected for the given recipient and donor ABO blood groups.

...
IgG was confirmed if the addition of antihuman IgG to the reduced wells resulted in a fourfold or greater increase in titer. The efficacy of washing to remove unbound IgG was confirmed in each assay by the addition of IgG sensitized Rh(D) positive erythrocytes (Coomb's control cells, strong, 50 μL, 3% suspension/well; Gamma Biologicals) to negative wells containing the highest serum concentration. Agglutination of sensitized RBCs indicated removal of extraneous IgG and confirmed the activity of the antiglobulin.

The absence of agglutination of O erythrocytes ruled out the presence of "irregular" hemagglutinating antibodies. A standard reference O serum was run with each assay to ensure comparability of results. Titers were adjusted according to the known titer of the standard. These corrections were never more than one twofold step.

Statistical analysis. Statistical analysis was performed using SYSTAT. Hemagglutinating antibody titers were approximately log-normally distributed as determined by probability plots (SYSTAT GRAPH module). Accordingly, log transformations were made before the calculation of descriptive statistics or application of parametric analyses. Arbitrary twofold units were used, where 1 represents a titer of 1/4, 2 a titer of 1/8, etc. The generalization for this relationship is: reciprocal titer = 2\(^{(-x)}\) x 4, where x is the titer in arbitrary twofold units and 4 is the reciprocal initial serum concentration. For parametric analyses (means, Student's t-test, analysis of variance, linear regression) undetectable antibody levels (ie, titers less than 1/2) were treated as 1/2 (0 in twofold units). Analysis of variance (ANOVA) and linear regression were performed using the MGLH module; descriptive statistics and Student's t-test were performed using the STATS module. Pearson's chi-square was calculated in the TABLES module. Graphics were created using SYGRAPH.

RESULTS

Reference ranges for the hemagglutinin assay. The sensitivity and specificity of the assay were characterized with a series of 132 paired donor/recipient sera obtained before BMT. The distribution of donor and recipient titers by specificity and isotype are shown in Fig 1. IgM titers of both specificities were log-normally distributed. For both anti-A and anti-B antibodies, IgM was a better indicator of subject ABO phenotype than IgG. Of 75 sera expected to be positive for anti-A antibody, 74 (99%) had IgM titers ≥ 1/4 and 63 (84%) had IgG titers ≥ 1/4. Of 101 expected anti-B sera, 96 (95%) had detectable IgM titers and 63 (62%) had detectable IgG. The geometric mean reciprocal titers (minus and plus the sample standard deviations) were 45 (10, 196), 39 (5, 277), 31 (7, 127), and 11 (2, 60) for anti-A IgM, anti-A IgG, anti-B IgM, and anti-B IgG, respectively.

No "irregular" or inappropriate antibodies were detected in this series. None of 132 sera agglutinated O erythrocytes. Similarly, in none of 89 possible instances were inappropriate specificities detected (eg, anti-A antibody in an A type individual).

Transfusions. Packed RBCs contain less than 10 mL of plasma per transfusion unit and were therefore considered not to be of influence on hemagglutinin titers. Intravenously administered gamma globulins, plasma, and platelet transfusions have higher Ig content and therefore could conceivably affect hemagglutinin titers through passive antibody transfer. To determine whether antibody titers could be affected by transfusion history, we examined the frequency of blood product administration in the first 3 months after BMT, the period during which maximal blood product support was required. A total of 45 patients receiving ABO mismatched BMT were studied. Platelet transfusions were divided in two categories, first choice or washed platelets and second choice...
HEMAGGLUTININS IN LYMPHOCYTE-DEPLETED BMT

or platelets of unknown ABO phenotype. First choice platelets are usually of AB phenotype or of an ABO phenotype lacking the antidonor antibody in major ABO-incompatible BMT or the antirecipient antibody in minor ABO-incompatible BMT. For washed platelets, 95% of the plasma volume is replaced by saline. For some blood products, preparations were either of pooled donor origin or the ABO phenotype was not available retrospectively; these are designated “unknown.”

There were no significant differences in platelet transfusion history (both categories) between the CCE group, the non-CCE + CsA group, or the non-CCE + Cy group (Pearson chi-square $P \geq .111$, all comparisons). However, in the non-CCE + Cy group a significantly higher proportion of patients received plasma transfusions (7 of 9, 78%) than in the other two groups (5 of 36, 14%, $P = .001$).

To determine whether administration of unwashed platelets elicited a rise in hemagglutinin titer corresponding to the ABO phenotype of the platelet product transfused, we evaluated the correlation between platelet dose and the difference in titers (IgM and IgG) before and after platelet administration. Data from all recipients of ABO mismatched grafts were divided into three time intervals: 0 to 2 weeks, 2 to 4 weeks, and 4 weeks to 3 months after BMT (Fig. 2). Platelet dose and change in relevant antibody titer (IgM or IgG) were uncorrelated. The greatest changes in titer were observed in recipients of nonCCE grafts in the first 2 weeks after BMT.

The same methodology was used to look for a correlation between plasma dose and change in antibody titer. Because the ABO phenotype of plasma products was unknown, the influence of each product on anti-A and anti-B titers was evaluated (IgM and IgG). In agreement with the platelet data, no correlation was found ($n = 30, r = .065, P = .734$; and $r = .026, P = .892$ for IgM and IgG, respectively). In fact, the recipient of the greatest number of plasma transfusions (122 units from 2 to 3 months after BMT) had undetectable hemagglutinins (anti-A and anti-B, IgM and IgG) at both 1 and 3 months after BMT.

Five recipients of ABO-mismatched grafts (2 CCE, 3 nonCCE) received intravenous gamma globulins in the first 3 months after BMT in a dose ranging from 7 to 90 g. No effect of this product could be detected in any of these five patients.

Fig 2. Lack of correlation between platelet dose and change in relevant hemagglutinin titer after and before platelet administration. All platelets were of known blood type and were suspended in plasma. Changes in titer are expressed in twofold steps. (C) Changes in titer measured between time 0 and 2 weeks; (D) 2 to 4 weeks; and (E), 4 weeks to 3 months. To prevent data points from overlapping, a small uniform random error (jitter) was introduced to the location of each point. The parameters of the least squares linear regression are indicated. Exact (nonjittered) values were used for the regression analyses. The lines of best fit are shown.

Major ABO incompatibility: Recipient-derived antibody. In major ABO mismatch, antidonor type antibody is of recipient origin and either represents preformed antibody or is the product of persistent host origin B cells (Table 1). When the donor is A or B and the recipient is O, a second specificity may be produced, but its origin cannot be uniquely ascribed to either recipient or donor. In the case of major plus minor ABO mismatch (eg, A into B), recognition occurs in both directions (donor antirecipient and recipient antidonor). Therefore, a unique donor specificity may also occur.

Because major mismatched ABO specificities can be ascribed uniquely to the recipient, analysis focused on the change in titer relative to pre-BMT levels. In all patient groups, antidonor antibody fell as a function of time after BMT (Fig 3). The magnitude of antibody decrease in the first 3 months after BMT did not differ between groups.
Fig 3. Course of antidonor hemagglutinins in major AB0 mismatched BMT. Results are plotted for individual recipients grouped by graft type and GVHD chemoprophylaxis. Reciprocal titers are shown on a log scale where negative samples (ie, titer <1/4 are indicated as 1. (O), Anti-A and (Φ), anti-B antibodies. The locations of overlapping symbols were shifted slightly.

(P > .2, Student's t-test, all comparisons). Neither was the proportion of patients with persistent recipient derived antibody (IgM or IgG, ≥3 months after BMT) significantly different between groups (P = .176, Pearson Chi-square test). One of the CCE patients demonstrated persistently high IgM and IgG antidonor antibody titers up to 1 year after BMT. Among all patients receiving nonelutriated grafts, 5 of 11 patients with an available serum sample at 6 months still had detectable antibody titers at that time point (IgM, IgG, or both). In no case could antidonor antibody be found at 1 year after BMT.

Minor AB0 incompatibility: Donor-derived antibody. In minor ABO mismatch, antibody specificities can be detected that are uniquely attributable to donor B cells (Table 1). In none of the seven patients who received an elutriated minor ABO-incompatible graft could an antirecipient antibody be detected, either early (2 to 4 weeks, 14 observations) or late after BMT (3 to 12 months, 13 observations) (Table 2). In 8 of 14 patients in the minor ABO incompatible, nonelutriated group, a transiently detectable antirecipient antibody was found early after BMT (28 observations). Donor-derived antibody was IgM, IgG, or both and was independent of GVHD chemoprophylactic regimen. Anti-A was detected in 8 of 13 possible instances (62%), and anti-B in 0 of 2 possible instances. Antirecipient antibody, when detected, was short-lived; none of the 14 patients had detectable titers 3 months or later after BMT (19 observations).

Matched specificities: Antibodies derived from the donor, recipient, or both. In certain ABO-mismatched combinations, antibody of a given specificity can be made by both donor and recipient, and thus cannot be uniquely attributed to either. In this respect they are comparable with antibodies elicited in ABO-matched BMT (Table 1). In the present analysis, these are referred to as donor and/or recipient-derived (DOR) antibodies. The effect of graft type (CCE or nonCCE), GVHD chemoprophylaxis (CsA or Cy), or AB0 match (match or mismatch) on DOR antibody was determined at intervals before and after transplant by ANOVA. At no time interval did GVHD chemoprophylaxis or AB0 match exert a significant influence on DOR titer (IgM or IgG, all P values >.176). The DOR titers are shown as a function of time after BMT in Fig 4. Early after BMT (2 to 4 weeks), recipients of nonCCE grafts had significantly higher DOR IgM titers compared with the CCE group (113 observations in 26 CCE and 31 nonCCE patients, P = .001 by ANOVA). Although not statistically significant, a similar effect was seen in DOR IgG titers. Graft type did not exert a significant effect on DOR IgM or IgG titers at other time intervals. In both graft types, the time to maximal DOR IgM titer preceded the time to maximal IgG titer. Maximal DOR titers (IgM and IgG) occurred later in recipients of elutriated grafts.

To assess the possibility that passive transfer of antibody present in the graft could account for the higher DOR titers observed in recipients of nonCCE grafts, the change in DOR
HEMAGGLUTININS IN LYMPHOCYTE-DEPLETED BMT

Table 2. Antirecipient Antibody Early After Minor ABO Incompatible BMT

<table>
<thead>
<tr>
<th>UNIQ*</th>
<th>Specificity†</th>
<th>IgM/lgG‡ (2 wk)</th>
<th>IgM/lgG‡ (4 wk)</th>
<th>Patients With Antirecipient ab/Total No.§</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCE</td>
<td>4 x Anti-A</td>
<td>&lt;4/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td>NonCCE + CsA</td>
<td>Anti-A</td>
<td>&lt;4/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Anti-B</td>
<td>128/512</td>
<td>32/256</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Anti-A</td>
<td>&lt;4/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Anti-B</td>
<td>8/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Anti-A</td>
<td>&lt;4/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Anti-A</td>
<td>&lt;4/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td>857</td>
<td>Anti-A</td>
<td>&lt;4/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td>969</td>
<td>Anti-A</td>
<td>&lt;4/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td>NonCCE + Cy</td>
<td>Anti-B</td>
<td>&lt;4/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Anti-A</td>
<td>4/8</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Anti-A</td>
<td>&lt;4/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Anti-A</td>
<td>&lt;4/8</td>
<td>&lt;4/8</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Anti-A</td>
<td>32/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
<tr>
<td>779</td>
<td>Anti-A</td>
<td>&lt;4/4</td>
<td>&lt;4/4</td>
<td>0/7</td>
</tr>
</tbody>
</table>

*Patient unique number.
†Expected specificity of donor-derived, antirecipient antibody.
‡Reciprocal titer of antirecipient antibody.
§Number of patients having titers equal to or greater than 1:4 (IgM or IgG).
| CCE: four patients with expected phenotype of anti-A and three patients with expected phenotype of anti-B; all had titers <1:4. |

antibodies from time 0 to 2 weeks after BMT was compared between recipients of ABO matched and mismatched non-CCE grafts. The grafts administered to the former group contained 600 to 700 mL donor plasma, whereas the latter contained little or no plasma. That no significant difference (IgM or IgG, P = .178 and .378, respectively) was observed indicates that the infusion of several hundred milliliters of plasma did not detectably affect hemagglutinin titer. In the absence of stable B-cell mixed chimerism, DOR antibody should be exclusively of donor origin by 1 year after BMT. To determine whether normal hemagglutinin levels were attained, we compared DOR titers 1 year after BMT with the pretransplant donor titers of relevant specificity. In the case of O into O transplants where both A and B specificities counted as DOR, both anti-A and anti-B titers for a given donor/patient pair were considered in the analysis. In recipients of both graft types DOR IgM was significantly lower than the relevant donor IgM titers (6.4-fold, n = 46, 4.

Fig 4. Course of DOR hemagglutinins in recipients of CCE and nonCCE ABO matched and mismatched grafts. Symbols represent mean titers, and bars indicate standard errors of the means. (Ø). NonCCE; (C), CCE. Mean titers are expressed in arbitrary twofold units (see Materials and Methods). (A) IgM, (B) IgG.
In contrast, late DOR IgG levels and the relevant donor IgG titers were more comparable in both groups (1.7-fold difference, $n = 46, P = .078$). Despite the similarity between mean group DOR IgG levels, individual late DOR IgG titers were highly correlated with those of their respective donors in recipients of nonelutriated, but not elutriated, grafts (Fig 5). In recipients of nonCCE grafts, the intercept ($-0.11 \pm 0.76$) and slope ($0.82 \pm 0.19$) of the linear least squares line of best fit are consistent with a direct correspondence between the magnitude of the pretransplant donor IgG hemagglutinin titer and that of the recipient late after BMT (Fig 5).

Irregular antibodies. None of 230 post-BMT patient sera agglutinated O erythrocytes. Similarly, in no case were inappropriate specificities detected (eg, anti-A antibody in an A or AB recipient with an A or AB donor).

DISCUSSION

Hemagglutinins provide an excellent model for studying the reconstitution of antibody responsiveness after BMT. They are directed against well-characterized oligosaccharide units attached to carrier molecules, are absent in infants, and are elicited by exposure to antigens homologous to the ABH specificities found on human erythrocytes. These ubiquitous determinants are also present on a variety of gram-negative bacterial strains which are regularly encountered by immunogenic routes, resulting in the generation of IgM, IgG, and IgA isotypes. Consistent with previous results, we found the prevalence of anti-B hemagglutinins somewhat lower than that of anti-A, in subjects of the relevant respective phenotypes (Fig 1).

Applied to the problem of immune reconstitution after BMT, the use of ABO-mismatched donor/recipient pairs allows one to distinguish between donor- and recipient-derived antibodies. Further, comparison of the generation of donor-derived responses in recipients of unmanipulated and elutriated grafts permits one to differentiate between donor-derived antibody responses conferred on the recipient by adoptive transfer of immune memory cells, and those generated de novo in the recipient environment.

Hemagglutinin titers are not significantly influenced by passive transfer of antibody present in blood products. This was conclusively demonstrated by the lack of correlation between platelet (Fig 2), plasma, and gamma globulin doses with changes in antibody titers during the first 3 months after BMT. Similarly, although significantly more patients in the nonCCE + Cy group received plasma transfusions than those in the nonCCE + CsA group, their hemagglutinin titers were not elevated.

Major ABO-incompatible BMT provided the opportunity to study the tempo of disappearance of recipient-derived antidonor antibody. This parameter is of concern because lymphocyte depletion could result in prolonged survival of host immunity, a phenomenon that has been suggested to account for the increased incidence of graft rejection associated with this procedure. The rate of disappearance of antidonor hemagglutinins is also of interest in itself, since significant immunohematologic problems have been associated with its persistence. Although the number of patients available for study was small, we found no evidence for prolonged survival of recipient-derived antibody in recipients of elutriated grafts. Only one recipient of elutriated marrow retained antidonor hemagglutinin by 1 year after BMT. Restriction fragment length polymorphism analysis of his

---

**Fig 5.** DOR IgG hemagglutinins in recipients of CCE (A) and nonCCE (B) grafts: Correlation of DOR hemagglutinin measured at 1 year after BMT (y axis) with pretransplant donor IgG titers of the relevant specificity (x axis). (○), Anti-A IgG; (●), anti-B IgG. The locations of overlapping symbols were shifted slightly. The parameters of the least squares linear regression are indicated. The lines of best fit and 95% confidence intervals are shown.
HEMAGGLUTININS IN LYMPHOCYTE-DEPLETED BMT

Peripheral blood showed mixed chimerism at that time. In recipients of nonCCE grafts, the use of CsA for AGVHD chemoprophylaxis may have enhanced survival of host origin antibody, but the small number of observations in the Cy group precluded a definitive comparison.

After minor ABO-incompatible BMT we found an early transient appearance of donor-derived, antirecipient antibody in 8 of 14 recipients of conventional bone marrow grafts. This contrasted sharply with recipients of elutriated grafts in whom we never detected this specificity. The transient appearance of antirecipient hemagglutinins in recipients of nonCCE grafts was not due to passive transfer because the plasma was removed from all minor ABO-incompatible grafts. Thus, it reflects the activity of mature donor lymphocytes infused with the nonelutriated graft. These data are consistent with our previous studies, which demonstrated that successful adoptive transfer of humoral- and cell-mediated responses to recall antigens required both donor-immune memory and early encounter with antigen in the recipient environment. Both of these conditions are fulfilled in the nonCCE group. Transient donor antirecipient hemagglutinins have been described previously in minor ABO incompatible conventional BMT. It has also been seen in minor ABO-incompatible solid-organ transplants where it has been attributed to passenger lymphocytes present in the graft. In contrast to our findings using elutriation as a means of lymphocyte depletion, investigators who used T-cell-specific monoclonal antibodies failed to abrogate the transient expression of donor-derived antirecipient hemagglutinins. Thus, elimination of this response in recipients of elutriated marrow most likely reflects the removal of B- as well as T-memory lymphocytes. Although it has been suggested that treatment with CsA favors the production of antirecipient antibody, our results and the results of others do not support this conclusion: antirecipient antibody was found in three of six patients who received Cy rather than CsA immunosuppression. In agreement with the results of others, we did not detect antirecipient antibody in any of our patient groups at 3 months or later after BMT. The disappearance of this specificity in recipients of nonCCE grafts, and its complete absence in recipients of CCE grafts, can be explained by the development of high-zone tolerance for the recipient ABO antigen due to its wide distribution in the tissues. Indeed, tolerance has been experimentally induced in O type infants by repeated parental challenge with purified A substance from birth to 8 months. The alternative explanation, that antirecipient antibody is produced but subsequently absorbed by recipient tissues, is less likely because this antibody was detected at maximal levels early after BMT when recipient ABO antigens are expressed on residual host erythrocytes as well as other tissues.

The existence of matched ABO specificities in ABO-mismatched BMT permits the immune reconstitution of this relatively limited population to be compared with that of recipients of ABO-compatible grafts. Although these matched (DOR) specificities cannot in theory be uniquely ascribed to donor or recipient, the kinetics of disappearance of recipient-derived antibody (Fig 3) suggest the donor origin of DOR antibody late after transplant. Early after transplant, DOR titers increased above pretransplant levels in both groups, with the rise in IgM titer preceding that of IgG. The early increase in IgM titer was far more pronounced in the nonCCE group, most likely reflecting the adoptive transfer of mature B- and T-memory lymphocytes in the nonelutriated grafts. This increase could not be attributed to passive transfer of antibody in the graft because DOR antibody was indistinguishable in the ABO-matched and -mismatched nonCCE groups, despite a substantially greater plasma volume in the nonCCE matched grafts. Late after BMT, both IgM and IgG levels decreased, but IgG levels remained comparable with donor pretransplant titers in both groups. The striking correlation between late DOR IgG in the nonCCE group, and the relevant pretransplant titers of their respective donors, suggests that even late after BMT, DOR IgG is still the product of adoptively transferred memory cells. That DOR IgG titers in the CCE group were of comparable magnitude, yet uncorrelated with individual donor titers, suggests the de novo generation of the immunologic components required for antigen-specific IgG response in this group.

The primary goal of this study was to determine the influence of graft lymphocyte depletion by CCE on the reconstitution of antibody responses. Taken together, these data bear on four important aspects: (1) the fate of recipient origin B lymphocytes; (2) the significance of memory lymphocytes present in the graft; (3) the contribution of T and B lymphocytes of donor origin differentiating within the new host environment; and (4) the induction of immunologic tolerance to host antigens. The removal of mature T and B lymphocytes from the graft did not result in a detectable prolongation of recipient-derived antibody production. However, it did entirely abrogate adoptive transfer of antibody uniquely attributable to the donor, and retarded the expression of DOR antibody. Despite this delay, recipients of lymphocyte-depleted and conventional allografts had comparable DOR IgG titers by 25 weeks after BMT. We have presented evidence suggesting that recipients of elutriated grafts generated this response de novo, while those who received unmanipulated grafts strongly suggests the induction of tolerance. That these specificities were never observed in recipients of elutriated grafts highlights the fact that such "autoimmune" responses are far less likely when the majority of immune cells have matured within the recipient environment.

ACKNOWLEDGMENT

The authors thank Sue Shirey MS MT (ASCP) SBB, The Johns Hopkins Blood Bank, Baltimore, MD, and Dr B.A. van Dijk and Ria Moors, University Hospital Nijmegen, The Netherlands, for their valuable advice on developing a quantitative hemagglutinin assay. We also thank Carole Scott for her excellent assistance in the preparation of this manuscript.
REFERENCES


Reconstitution of antibody response after allogeneic bone marrow transplantation: effect of lymphocyte depletion by counterflow centrifugal elutriation on the expression of hemagglutinins

BM Bar, GW Santos and AD Donnenberg

Updated information and services can be found at:
http://www.bloodjournal.org/content/76/7/1410.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml