Donor Immunization With Haemophilus influenzae type b (HIB)-Conjugate Vaccine in Allogeneic Bone Marrow Transplantation

By Deborah C. Molrine, Eva C. Guinan, Joseph H. Antin, Susan K. Parsons, Howard J. Weinstein, Catherine Wheeler, Carol McGarigle, Peter Blanding, Nichole R. Phillips, Karalyn Kinsella, Katherine Deans, Angela Ciamarra, Amy Goorin, Suzanne George, and Donna M. Ambrosino

Bone marrow transplant patients are at increased risk for infections with polysaccharide encapsulated organisms and respond poorly to polysaccharide vaccines. We evaluated the effect of donor immunization with Haemophilus influenzae type b (HIB) polysaccharide-conjugate vaccine on recipient antibody responses following allogeneic bone marrow transplantation. Thirty-two allogeneic transplant patients and their donors were immunized before transplantation with HIB-conjugate, tetanus toxoid and 23-valent pneumococcal vaccines. Following transplantation, patients received HIB-conjugate and tetanus toxoid vaccines at 3, 6, 12, and 24 months and 23-valent pneumococcal vaccine at 12 and 24 months. Thirty-three patients with unimmunized donors were immunized following transplantation in an identical manner. Patients whose donors were immunized had significantly higher total anti-HIB antibody concentrations at 3 months ($P = .0001$), 6 months ($P = .0001$), 12 months ($P = .0001$), and 24 months ($P = .002$) after transplant compared with patients whose donors were unimmunized. Higher antitetanus toxoid antibody concentrations were also noted in patients with immunized donors, where donor immunization with pneumococcal vaccine had no effect on antibody concentrations following transplantation. Donor immunization with HIB-conjugate vaccine resulted in higher antibody concentrations in patients as early as 3 months after allogeneic transplantation and may be an effective strategy to prevent HIB infections.

© 1996 by The American Society of Hematology.

MATERIALS AND METHODS

Study Population and Immunization Schema

Patients older than 2 years of age undergoing matched related allogeneic transplantation for lymphoma, leukemia, or aplastic anemia between April 1990 and August 1993 at Brigham and Women's and Children's Hospitals in Boston, MA were recruited. The 65 study patients were conditioned for allogeneic BMT according to diagnosis and current protocols; 49 received chemotherapy and total body irradiation, 16 received chemotherapy alone, and four patients underwent T-cell depletion as previously described.3 Graft-versus-host disease (GVHD) prophylaxis consisted of methotrexate and/or cyclosporine until day 50 posttransplantation and was then tapered per discretion of the attending physician.

Patients whose donors were available for immunizations 7 to 10 days before marrow harvest were assigned to the donor immunization group, and those whose donors were not available were assigned to the unimmunized donor group. In addition, patients not approached before transplantation were offered enrollment in the unimmunized donor group at their initial posttransplant visits.

BMT donors of patients assigned to the immunized donor group were immunized 7 to 10 days before marrow harvest with 0.5 cc Haemophilus influenzae type b (HIB)-conjugate vaccine (HiB TITER, Lederle-Praxis Biologicals, Pearl River, NY), 0.5 cc tetanus toxoid aluminum phosphate-adsorbed vaccine (Wyeth Laboratories, Philadelphia, PA), and 0.5 cc of 23-valent polysaccharide pneumococcal vaccine (Pnu-immune 23, Lederle-Praxis Biologicals) intramuscularly. BMT recipients in the immunized donor group were immunized 7 to 10 days before transplantation with the same vaccines.
Following transplantation, BMT patients assigned to either the immunized donor group or unimmunized donor group received HIB-conjugate and tetanus toxoid vaccines at 3, 6, 12, and 24 months and 23-valent pneumococcal vaccine at 12 and 24 months. Serum was obtained from donors at the time of immunization, at bone marrow harvest, and 3 to 6 weeks following immunization. Serum was obtained from each BMT patient before receiving immunizations at 3, 6, 12, and 24 months and 3 to 6 weeks following the final immunization at 24 months. Patients who had a scheduled visit between 12 and 24 months posttransplant had an additional serum sample obtained. All sera were stored at -70°C until assayed.

Data were analyzed after all patients had reached at least the 12 month immunization time point. Eight patients in the immunized donor group and two in the unimmunized donor group did not yet reach the 24-month time point at the time of analysis. In addition, six patients in the unimmunized donor group were not included in the post 24-month evaluation of pneumococcal responses, as these patients were offered an investigational pneumococcal vaccine. The study was approved by the Institutional Review Boards of participating institutions, and informed consent was obtained from enrolled donors, patients, or their guardians.

**Antibody Assays**

Total binding anti-HIB capsular antibody was measured by radioimmunoassay (RIA) using a standard Food and Drug Administration protocol with tritiated polyribosylribitol phosphate (provided by Dr. Porter Anderson, University of Rochester, NY). The RIA was standardized using the Center for Biologic Research and Review standard serum pool with an assigned value of 70 μg/mL. IgG anti-HIB, antitetanus toxoid, and antimeningococcal group A antibody concentrations were measured and standardized by enzyme-linked immunosorbent assay (ELISA) as previously described.

IgG antipneumococcal antibody was measured by amplified ELISA after absorption with cell wall polysaccharide. A reference plasma pool PSAB90 (FDA, Bethesda MD) was standardized based on a reference pool 89SF (FDA). We chose to measure three serotypes (6B, 9V, and 19F), as 6B is a particularly poor immunogen, whereas 9V and 19F are more immunogenic.

**Statistics**

Antibody concentrations were transformed to logarithms for statistical calculations. Antibody concentrations below the limit of assay sensitivity were assigned values of one half the lower limit. Comparisons of geometric means of antibody concentrations were performed by the two-tailed t-test for parametric analysis and by the Mann-Whitney rank sum test for nonparametric analysis. Paired analyses were done by the two-tailed t-test or Wilcoxon's signed-rank test. The frequency of individuals with protective concentrations of total anti-HIB antibody were compared by the Fisher exact test.

**RESULTS**

**Patient Population**

Of the 159 eligible patients transplanted during the study period, 87 patients were approached to participate: nine refused, and 78 patients were enrolled. Evaluable patients were defined as those who received immunizations and survived without relapse until at least 3 months following BMT. Individuals who missed immunizations, relapsed, or died after 3 months were included in the analysis until the time these events occurred. Immunizations were considered missed if a patient was not immunized within 21 days of their 3-month or 6-month posttransplant date or within 45 days of their 12- or 24-month posttransplant date. Forty-four patients were enrolled in the immunized donor group and 32 were evaluable (nine died or relapsed before 3 months, two missed immunizations, and one transplant was postponed). Thirty-four were enrolled in the unimmunized donor group and 33 were evaluable (one patient missed the 3-month blood draw and relapsed before 6 months). Table 1 details the clinical characteristics of the evaluable patients at each time point.

**Donor Responses to HIB-Conjugate, Tetanus Toxoid, and Pneumococcal Vaccines**

Donors had a 99-fold rise in geometric mean total anti-HIB antibody from 1.44 μg/mL before to 142 μg/mL following immunization with HIB-conjugate vaccine (P < .0001) (Table 2). Geometric mean antibody concentrations following tetanus toxoid and pneumococcal immunizations were also significantly increased with geometric mean fold rises of 6.45 and 3.24, respectively. Sera at the time of bone marrow harvest were available on 20 of the 32 donors. Antibody concentrations at the time of harvest (mean 8.5 days following immunization) were similar to those determined 3 to 6 weeks following immunization (Table 2). There was no increase in the geometric mean antibody concentration to a control antigen, meningococcal group A polysaccharide.

**Effect of Donor Immunization on Antibody Concentrations Following BMT**

Of the 159 BMT patients in the immunized donor group had higher geometric mean total anti-HIB antibody concentrations following transplantation...
Table 2. Antibody Responses of Allogeneic BMT Donors to HIB-Conjugate, Tetanus Toxoid, and Pneumococcal Vaccines

<table>
<thead>
<tr>
<th>Antibody to:</th>
<th>Pre Harvest (n = 30)</th>
<th>Postharvest (n = 20)</th>
<th>Fold-Rise*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total HIB (by RIA)</td>
<td>1.44</td>
<td>199</td>
<td>142§</td>
</tr>
<tr>
<td>Tetanus toxoid IgG</td>
<td>63.25</td>
<td>420</td>
<td>401§</td>
</tr>
<tr>
<td>Pneumococcal IgG, Mean</td>
<td>1.92§</td>
<td>5.46</td>
<td>6.40§</td>
</tr>
<tr>
<td>Meningococcal group A‡</td>
<td>9.37</td>
<td>10.4</td>
<td>Not Done</td>
</tr>
</tbody>
</table>

*Postharvest antibody concentrations divided by preimmunization concentrations except for meningococcal group A polysaccharide that divides harvest antibody concentrations by preimmunization concentrations.

†The average of antibody concentrations measured for serotypes 6B, 9V, 19F.

‡Control antibody.

§P = .0001 by t-test on paired data comparing postharvest to preimmunization antibody concentrations.

as compared with patients with unimmunized donors (Fig 1). The differences reached significance at 3 months (P = .0001), 6 months (P = .0001), 12 months (P = .0001), post 12 months (P = .002), and 24 months (P = .002) following BMT (Table 3). Geometric mean IgG anti-HIB antibody as measured by ELISA was also significantly higher in patients of the immunized donor group (Table 3). The measurements of HIB antibody by these two assays were highly correlated for each time point (r = 0.65 to r = 0.97).

Active responses to HIB-conjugate vaccine were evaluated by comparing individual patients' antibody concentrations before and after each immunization (Table 4). To assess responses to the 3-month dose, antibody concentrations measured at 6 months were compared with 3 month values. To assess responses to the 6-month dose, 12-month concentrations were compared with 6 months. Responses to the 12- and 24-month doses were determined using the concentrations measured in the post 12- and 24-month immunization sera, respectively. For patients in the immunized donor group, total anti-HIB antibody concentrations increased significantly following the 3-month dose (P = .044), 12-month dose (P = .004), and 24-month dose (P = .001). In contrast, BMT patients in the unimmunized donor group had no increase in antibody concentrations until after the 12-month dose and a significant increase only after the 24-month dose (P = .001) (Table 4).

To determine the potential clinical impact of donor immunization, we compared the number of patients who reached "protective" levels of anti-HIB antibody in the two groups. A concentration of 1 µg/mL of total anti-HIB antibody is considered predictive of long-term protection.24 A significantly higher percentage of patients in the immunized donor group reached protective antibody concentrations of total anti-HIB antibody at 3 months (97%), 12 months (92%), and 24 months (92%) as compared with those patients with unimmunized donors (55%, 37%, and 47%, respectively) (Table 5).

Responses to tetanus toxoid vaccine. The effect of donor immunization with tetanus toxoid vaccine on recipient antibody responses was similar to that seen with HIB-conjugate vaccine. Geometric mean IgG antitetanus toxoid antibody concentrations were higher in the immunized donor group at all measured time points reaching significance at 6 and 12 months following transplantation (Fig 1, Table 3). Responses to doses administered following transplantation occurred earlier in the immunized donor group as compared with those in the unimmunized donor group (Fig 2).

Responses to unconjugated pneumococcal vaccine. Donor immunization with 23-valent polysaccharide pneumococcal vaccine did not result in significantly higher antibody concentrations in recipients following BMT (Table 3). Antibody concentrations were similar at 3 months and declined over time in both the immunized and unimmunized donor groups with no response noted to the 12-month dose. Pneumococcal antibody concentrations increased slightly after the 24-month dose and were not significantly different between the immunized donor and unimmunized donor groups (Table 3). Following the 24-month pneumococcal immunization, geometric mean IgG pneumococcal antibody concentrations for the three measured serotypes 6B, 9V, and 19F were 0.26

Fig 1. The effect of donor immunization on total anti-HIB and IgG antitetanus toxoid antibody concentrations following transplantation. (■) Represent geometric mean antibody concentrations of patients whose donors were immunized with HIB-conjugate and tetanus toxoid vaccines before marrow harvest. (○) Represent geometric mean antibody concentrations of patients whose donors were unimmunized. (-----) Represents presumed protective level of total anti-HIB antibody. Arrows indicate the time when vaccine doses were administered to all patients following transplantation.
Factors Influencing Recipient Antibody Concentrations Following BMT

To determine if factors other than donor immunization affected antibody concentrations following BMT, we examined in a multiple stepwise linear regression analysis the following variables: donor immunization status, underlying diagnosis, sex, recipient age, donor age, T-cell depletion, total body irradiation, use of intravenous immunoglobulin (IVIG) within 30 days of immunization, presence of GVHD at the time of immunization, and treatment for GVHD with steroids or other immunosuppressive agents within 30 days of immunization. Because donor age and recipient age were highly correlated in our study population (r = 0.79), we chose to enter donor age into the regression model when both variables were significant.

Donor immunization was consistently independently associated with higher total anti-HIB antibody concentrations in BMT patients at 3 months (P = .0001), 6 months (P = .0001), 12 months (P = .0005), post 12 months (P = .019), and 24 months (P = .005) following transplantation. Younger donor age was also independently associated with increased total anti-HIB antibody concentrations at 6 months (P = .065), 12 months (P = .015), post 12 months (P = .004) by t-test on paired data comparing pre to postantibody concentrations.

Table 4. Effect of Donor Immunization on Active Responses to HIB-Conjugate Vaccine Following Allogeneic BMT

<table>
<thead>
<tr>
<th>Vaccine Dose</th>
<th>Immunized Donor Group</th>
<th>Unimmunized Donor Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>3 mo</td>
<td>6.96†</td>
<td>19.2†</td>
</tr>
<tr>
<td>6 mo</td>
<td>23.1†</td>
<td>27.4†</td>
</tr>
<tr>
<td>12 mo</td>
<td>19.0†</td>
<td>82.1†</td>
</tr>
<tr>
<td>24 mo</td>
<td>48.9†</td>
<td>225†</td>
</tr>
</tbody>
</table>

† Postimmunization antibody concentrations divided by preimmunization concentrations of patients with paired sera available before and after each vaccine dose.
‡ P = .004 by t-test on paired data comparing pre to postantibody concentrations.
§ P = .004 by t-test on paired data comparing pre to postantibody concentrations.
¶ P = .001 by t-test on paired data comparing pre to postantibody concentrations.
There was a strong correlation between donor age and total anti-HIB antibody concentrations of donors measured at the time of harvest with younger donors having higher concentrations ($r = -0.69$, $P = .001$) (Fig 2).

**DISCUSSION**

Patients whose donors had been immunized with HIB-conjugate vaccine had significantly higher anti-HIB antibody concentrations following allogeneic transplantation as compared with patients with unimmunized donors. Similar results were noted for tetanus toxoid vaccine with higher antibody concentrations for those patients with immunized donors. In addition, active responses to HIB-conjugate vaccine were noted as early as 3 months following transplantation in patients with immunized donors and not until 12 to 24 months in the group with unimmunized donors. As a result, a higher percentage of patients in the immunized donor group had “protective” concentrations of total anti-HIB antibody during the first 2 years posttransplantation.

Previous studies with tetanus toxoid and *Pseudomonas aeruginosa* vaccines have also shown that donor immunization increases antibody concentrations in BMT patients. Wimperis et al demonstrated that immunizing both donor and recipient with tetanus toxoid before transplant resulted in higher antibody concentrations after BMT than immunizing either donor or recipient alone. This suggests that activated B cells secreting antibody are transferred to BMT recipients who host antigen presenting cells can further expand the B-cell response. Furthermore, donor memory B and T helper cells are probably transferred that prime recipients for responses to vaccine doses administered following transplantation. Our study demonstrates that donor immunization with a capsular polysaccharide-conjugate vaccine also primes BMT patients for early responses.

In contrast to HIB polysaccharide-conjugate vaccine, unconjugated pneumococcal vaccine administered to donors did not significantly increase recipient antibody concentrations following BMT. We suggest that donor immunization failed to enhance responses to pneumococcal vaccine because unconjugated polysaccharides are T-cell independent antigens that do not prime for booster responses. An alternative explanation for our results is that differences in the immunization schedule were responsible for the lack of effect of donor immunization with pneumococcal vaccine. Due to concerns of local reactions to multiple doses, patients were immunized with only two doses of pneumococcal vaccine as compared with four doses of HIB-conjugate vaccine following transplantation.

Other investigators have suggested that HIB-conjugate vaccine administered after BMT will produce adequate responses without donor immunization. Their study reported on 20 patients who received two doses of HIB-T conjugate vaccine (Pasteur Mérieux Connaught, Paris, France) 4 to 80 months after BMT. Although the study group included some patients immunized early after transplant, mean time of immunization was 16.9 months. We have also previously reported that two doses of HIB-conjugate vaccine administered late after BMT (at 12 and 24 months) produce reliable responses. The present study documents that donor immu-
zation resulted in higher HIB antibody concentrations at 3 and 6 months posttransplant and significantly increased the number of BMT patients who achieved early protective responses.

In addition to the effect of donor immunization on recipient antibody concentrations, we noted that younger age was consistently independently correlated with higher concentrations to HIB and tetanus toxoid following BMT. We were not able to differentiate the effect of donor age from recipient age because sibling donors and recipients were of similar ages (r = +0.79). We speculate that donor age is the important factor, as it was strongly correlated to donor antibody concentrations which, in turn, were correlated to antibody concentrations in recipients. We propose that younger donors had higher antibody responses, as they would have been primed more recently with diphtheria toxoid, the protein carrier for HIB-conjugate vaccine, and tetanus toxoid from routine childhood immunizations. However, it is also possible that recipient age may influence antibody responses following transplantation, as it has been demonstrated that the recovery of naive CD4+ T lymphocytes following chemotherapy is increased in younger children and correlates to thymic function.21 In addition, naive CD4+ T-cell recovery has been shown to be enhanced in young children following BMT.24 Enhanced T-cell recovery would presumably result in improved responses to vaccines.

A limitation of our study is that patients were not randomized but assigned to immunization groups based on the availability of donors before marrow harvest. Our results could be biased if those entered into the immunized donor group were inadvertently selected to be “better responders”; however, clinical characteristics of the two groups were similar. Of note, the percentage of patients who received IVIG at 3 months was higher in the immunized donor group, though the use of IVIG was not significantly different at 6 and 12 months after transplant and none of the evaluable patients in either group were receiving IVIG at 24 months. In addition, we measured meningococcal group A polysaccharide to control for passive antibody, and no differences in antibody concentrations were noted at 3, 6, and 12 months after transplant between the two groups. Multivariate regression analyses were also performed to examine the effect of clinical variables on anti-HIB antibody concentrations. Donor immunization was strongly independently associated with increased antibody concentrations to HIB when IVIG and all other factors known to affect antibody concentrations were included in the regression analyses. It is unlikely, therefore, that the differences in antibody results to HIB immunization between the immunized donor and unimmunized donor groups were due to passive antibody from the receipt of IVIG. Interestingly, GVHD was not significantly independently associated with lower anti-HIB or antitetanus toxoid antibody concentrations, which might have been expected, as these patients were receiving immunosuppressive therapy for their disease. The small number of patients with chronic GVHD may have limited our ability to see an effect. Finally, similar to their donors, recipient antibody concentrations increased most dramatically to HIB-conjugate vaccine, followed by responses of lesser magnitude to tetanus toxoid and pneumococcal vaccines. These observations support that differences in antibody concentrations between the groups were due to donor immunization.

We conclude that donor immunization with HIB-conjugate vaccine combined with pretransplant recipient immunization followed by additional doses at 3, 6, 12, and 24 months post-BMT will result in protective antibody concentrations throughout the posttransplant period. With the recent development of pneumococcal-conjugate vaccines, the strategy of donor immunization could have a substantial clinical impact on pneumococcal disease in this high-risk population. We are now planning to extend these results to study donor immunization in a randomized trial with an investigational 7-valent pneumococcal conjugate vaccine.29 Finally, donor immunization may be an effective strategy to prevent other infections in BMT patients. For example, herpes virus reactivation is a major problem following BMT. We suggest that investigational inactivated varicella zoster virus and cyto-megalovirus vectorized vaccines are good candidates for initial studies evaluating donor immunization for prevention of viral reactivation.30,31

REFERENCES

11. Friedland IR, McCracken GH, Jr: Management of infections