Immunity Against *Pseudomonas Aeruginosa* Adoptively Transferred to Bone Marrow Transplant Recipients


Infection is a common problem for bone marrow transplant (BMT) recipients during the period of neutropenia that immediately follows the procedure. Gram-negative infections present a particular hazard in these immunocompromised hosts. To augment host defenses against one such pathogen, *Pseudomonas aeruginosa*, we immunized bone marrow transplant donors and/or recipients with a polyvalent O-polysaccharide-toxin A conjugate vaccine. When either donor or recipient alone was vaccinated before transplant, no increase in specific antibody titers to any of the vaccine components was observed in the recipient. However, when both donor and recipient were vaccinated before transplant, increases in antibody titers to all polysaccharide components occurred to levels shown to be protective in animal models of gram-negative sepsis. Specific antibodies were primarily of the IgG, and IgG₄ subclass even though IgG₁ subclass deficiency is common after BMT. The requirement for both donor and recipient immunization reflects the need for primed donor B lymphocytes in the marrow inoculum to be transferred into an antigen-containing environment so that maximum B-cell proliferation and antibody secretion can occur. Adoptive transfer of antibody responses to *Pseudomonas aeruginosa* and other common bacterial pathogens has the potential to reduce infection-related morbidity and mortality after allogeneic bone marrow transplantation.

**MATERIALS AND METHODS**

**Patients.** Fourteen patients (eight male, six female) underwent allogeneic bone marrow transplant (BMT) from human leukocyte antigen (HLA) identical mixed lymphocyte culture (MLC) unreactive siblings. Nine patients were transplanted for acute myeloblastic leukemia (AML) in first complete remission, four for acute lymphoblastic leukemia (ALL) in first complete remission, and one for chronic myeloid leukemia (CML) in chronic phase. Median age was 20 years (range 12 to 42 years). All patients received conditioning with cyclophosphamide 120 mg/kg intravenously (IV) followed by single fraction total body irradiation (7.5 Gy) before infusion of allogeneic marrow. One patient also received busulphan 2.6 mg/kg × 8 orally. Five patients were treated with Campath immunoglobulin (Ig)G monoclonal antibody to CD5 antigen on mature T cells conjugated to the A chain of ricin. Based on data from previous studies, and on the appearance of anti-*Pseudomonas* antibody (Ab) 1 week after immunization of normal donors with a peak at 3 to 4 weeks, patients and/or donors were vaccinated once on the same day, between 7 and 10 days before transplant. In six pairs, both donor and recipient were immunized (D,R); in four, only the donor was immunized (D,R₀); and in four, only the recipient received the vaccine (D,R₁) (Table 1). All but one patient (D,R₁ group) survived the 90 days post-BMT over which the study took place. No patient in any group developed *Pseudomonas* colonization/infection. No patient received routine IV Ig.

*Pseudomonas* vaccine. The *P aeruginosa* conjugate vaccine consists of oxidized polysaccharides from eight *P aeruginosa* lipopolysaccharide serotypes (termed IT 1-5, IT-2, and HAbs 3 & 4) conjugated to purified *P aeruginosa* toxin A. Isolation and purification of polysaccharide and toxin A and preparation of the conjugate vaccine are as previously described. Following written informed consent, and using a protocol approved by the Hospital Ethical Practices subcommittee, vaccination into the deltoid region of the upper arm was performed using the deep subcutaneous route. Enzyme-linked immunosorbent assay (ELISA). ELISA for detection of antibodies to vaccine components was performed as previously described. In brief, wells of Dynatech Immulon (Buchs, Switzerland) microtiter plates were coated with 100 μL of a solution of the individual antigens in carbonate buffer. Plates were incubated at room temperature overnight, washed 3 times in PBS-Tween, and 100 μL of appropriate dilutions of the test sera in phosphate-buffered saline (PBS) Tween was added into triplicate wells. Plates were
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Table 1. Patient Vaccination Details

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Sex</th>
<th>Diagnosis</th>
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<td>AML M1 ICR</td>
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</tr>
<tr>
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<td>F</td>
<td>AML M3 ICR</td>
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<tr>
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<td>AML M4 ICR</td>
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<tr>
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<td>M</td>
<td>C ALL ICR</td>
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<tr>
<td>42</td>
<td>M</td>
<td>AML M2 ICR</td>
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<tr>
<td>26</td>
<td>F</td>
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<td>F</td>
<td>C ALL ICR</td>
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<td>F</td>
<td>AML M4 ICR</td>
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</tr>
<tr>
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<td>M</td>
<td>C ALL ICR</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
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<td>T ALL ICR</td>
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</tr>
<tr>
<td>41</td>
<td>F</td>
<td>GCL ICP</td>
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</table>

*Donor alone immunized 7 to 10 days pre-BMT.
†Recipient alone immunized 7 to 10 days pre-BMT.
‡Donor and recipient immunized 7 to 10 days pre-BMT.
§GVHD was treated with short course methylprednisolone 1-10 mg/kg.

incubated at 22°C for 3 hours, washed 3 times and a 1:2500 dilution of peroxidase labeled goat anti-human IgG (Kirkegaard & Perry Lab, Gaithersburg, MD) added to all wells. After incubation and washing, 100 µL of the anti-human IgG subclass antibody was added. Subclass antibodies and dilutions were as follows: anti-IgG1 (Unipath clone NL 16) 1:4000; anti-IgG2 (Binding Site clone H6015) 1:2000; anti-IgG3 (Binding Site clone H6050) 1:1000; anti-IgG4 (Binding Site clone H6023) 1:2000. Plates were incubated overnight, further washed and a 1:2500 dilution of peroxidase-conjugated goat anti-mouse IgG (Nordic Immunology, Tilbury, The Netherlands) added to each well. After incubation, color was developed with 100 µL of ABTS substrate solution. The OD₅₆₂ was determined after 30 minutes of incubation at 22°C. ELISA units were calculated by multiplying OD₅₆₂ values obtained within the linear portion of the dilution curve by the serum dilution.

RESULTS

Immunization with the *P aeruginosa* conjugate vaccine was well tolerated. No local or systemic reactions were noted in either transplant recipients or their marrow donors. When recipients or marrow donors alone were given the vaccine pretransplant, there was little antibody response to any vaccine component in the recipients during the post-BMT period (Fig 1A and B, Fig 2A and B). Immunization of both

![Graph A](image1)

**Fig 1.** Serum antibody responses to polysaccharide serotypes of the octavalent *P aeruginosa* vaccine in BMT recipients. Antibody responses to the immunotype 3 (IT-3) of the vaccine are illustrated. Four D,R₀ patients (A), four D,R_I patients (B), and six D,R₂ patients (C) (both donor and recipient vaccinated) are illustrated. BMT took place 7 to 10 days after the immunization (week 1 to 1.5).
donor and recipient 7 to 10 days before transplant allowed adoptive transfer to occur. Figures 1C and 2C show a representation of the kinetics of the antibody response; Table 2 shows Ab levels to the different components of the vaccine in normal donors compared with D,R, recipients. Although responses in normal donors were greater, the D,R, patients still had IgG antibody levels to all eight LPS serotypes contained in the vaccine that were substantially higher (approximately 3-to-20-fold) postvaccination. The weakest immune response was seen for the two serotypes (IT-6 and HAbs 3) with the highest baseline levels of antibody. No comparable rise was noted to the toxin A moiety of the vaccine. Peak anti-LPS IgG antibody levels were observed 2 to 4 weeks posttransplant and remained elevated up to 3 months following BMT (Fig. 1C, 2C). No rise in IgM antibodies was detected in donors or recipients.

Antibodies of the IgG2 subclass appear to be critical in conferring protection against infections caused by bacterial pathogens expressing serospecific polysaccharide surface antigens, and prolonged deficiency of the IgG2 subclass is

<table>
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<tr>
<th>Vaccine Component</th>
<th>Prevaccine</th>
<th>Normal Donors</th>
<th>Postvaccine</th>
<th>Prevaccine</th>
<th>BMT Recipients</th>
<th>Postvaccine</th>
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<td>5.3</td>
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<td>48†</td>
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<tr>
<td>HAbs 4</td>
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<td>68†</td>
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<td>1.6</td>
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</tr>
</tbody>
</table>

Data shown are from nine normal donors and six recipients who were vaccinated along with their allogeneic donors 7 to 10 days pretransplant. Postvaccine samples were obtained 28 days after immunization.

*P < .01 for significant difference pre- and postvaccine.
†P < .005.
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common after BMT.24 We therefore determined the IgG subclass distribution of adoptively transferred anti-*P. aeruginosa* LPS antibody in two D,R, BMT recipients (Fig 3). IgG2 was the predominant subclass followed by IgG1. In these patients, elevated levels of IgG2 and anti-*P. aeruginosa* LPS antibody persisted for at least 3 months posttransplant (data not shown).

**DISCUSSION**

The procedure of allogeneic bone marrow transplantation provides the possibility of transferring antibody responses from an immunized marrow donor to a nonimmune recipient. We have shown that IgG antibody responses induced by a *P. aeruginosa* vaccine can be adoptively transferred to allogeneic bone marrow transplant recipients. When both donors and recipients were vaccinated 7 to 10 days pretransplant, levels of IgG antibody to polysaccharide components of the vaccine were substantially increased over baseline levels within 2 to 3 weeks of vaccination. They remained elevated for 5 weeks or longer, during the period of neutropenia when there is increased susceptibility to overwhelming infection. Marked increases in specific antibody to the polysaccharide component of the vaccine were produced and the duration of response was equivalent to that found when normal individuals are immunized. On day 28, 50% develop peak Ab levels that fall to 40% to 75% of maximum by 56 to 70 days.17,18,20 The antigens recognized, the isotype distribution, and the magnitude of the immune response also correlate well to the pattern of antibody production previously seen in normal individuals.18 Antibody elicited by these vaccinations has been shown to reduce fatal episodes of wound sepsis in a passive protection animal model.30 Prior studies suggest the level of anti-LPS antibody engendered in BMT patients should prove to be equally protective.25,26

Consistent adoptive transfer of high titer antibody responses required immunization of both donor and recipient before transplant. Studies with other antigens have shown that during the first 1 to 3 weeks following donor immunization, specific antibody-secreting B cells appear in the blood and can be transferred with the donor marrow inoculum. However, these Ab-secreting cells are short-lived and must be subsequently exposed to antigen in the recipient for continued proliferation, differentiation and antibody secretion to occur.21 These IgG responses are made at a time when few CD4 positive T cells are detected in the recipient; we have suggested that helper effects are produced instead by CD3 negative large granular lymphocytes that secrete B-cell growth and differentiation factors and circulate in large numbers in the immediate posttransplant period.17

Adoptively transferred B cells secrete high levels of IgG1 antibody, an isotype important in protection against pathogens with polysaccharide surface antigens. It is paradoxical that BMT recipients can mount a high titer, adoptively transferred IgG1-specific response at a time when total IgG levels are falling.24 These observations imply that deficits in IgG1 production are not due to failure of transfer of IgG1-secreting precursor cells but to abnormalities of those antigen-presenting cells or T lymphocytes responsible for the selection and expansion of previously unstimulated IgG1-producing B cells in the host. Poor IgM responses to these vaccines are made in normal donors, perhaps because the responses observed are of the "recall" type.25 Most adults have baseline antibody titers to *Pseudomonas >1 μg/mL* presumably because of exposure to antigenic stimulation from bacteria present in food and water.18 There is no evidence for any specific IgM response in the recipients, an observation consistent with previous studies in which specific IgM responses are not adoptively transferred in man even when IgM responses are made by the donors.21 A number of explanations for this result have been suggested.21 IgM transfer does not appear to be necessary to confer protection; evidence from animal models suggests that IgG responses—of appropriate subclass distribution—are sufficient for protection from the adverse effects of gram-negative organisms.1,18

In the individuals studied, the *P aeruginosa* vaccine was safe. No adverse reactions were seen in either the immunocompromised patients or in their normal donors. In a much larger study of immunization of normal volunteers, the only adverse reactions reported were of mild erythema and discomfort localized to the site of injection.20 This is in marked contrast to prior studies evaluating an earlier LPS-based vaccine that elicited clinically significant adverse reactions in about 50% of immunocompromised vaccinees.26,28 In addition, immunization with this LPS-based vaccine for the most part engendered only a modest, transient antibody response, primarily of the IgM class, and required serial vaccinations.

The patient cohort studied here was too small to obtain efficacy data, but the ability of *Pseudomonas* sp. to cause life-threatening disease in the immunocompromised host is well documented.1-10,30 Although this organism is not the most common cause of infection after BMT, *Pseudomonas* septicemias or soft-tissue involvement consistently produce severe morbidity and a high mortality. The organism continues to account for up to 10% of procedure-related deaths after BMT and to produce significant morbidity in 5% to 15% of all BMT recipients.1-10 Enhanced immunity induced by

**Fig 3.** IgG subclass distribution in two D,R, patients following immunization with *P. aeruginosa* vaccine. Results are shown for antibody to Habs 4, IT-3 and IT-4 components of the vaccine. Results from serum taken at the peak of antibody responses (4 weeks posttransplant) is illustrated and is representative of subclass distribution at 3 and 8 weeks following BMT.
adoptive transfer of primed lymphocytes from immunized donors should therefore impact on posttransplant morbidity and mortality. Cost of the vaccine is low, making savings in both human and financial terms likely to far outweigh the small investment in time and vaccine required for pretransplant immunization. Moreover, similar vaccines derived from other gram-negative bacterial pathogens such as Klebsiella spp. and Escherichia coli have recently become available.\textsuperscript{11,12} It is therefore now feasible to design randomized trials immunizing donor/recipient pairs with all three vaccines in combination to determine whether adoptive transfer of antibody responses to some of the most important bacterial pathogens in the neutropenic patient will significantly reduce overall morbidity and mortality from posttransplant gram-negative sepsis.

REFERENCES


Immunity against Pseudomonas aeruginosa adoptively transferred to bone marrow transplant recipients

DJ Gottlieb, SJ Jr Cryz, E Furer, JU Que, HG Prentice, AS Duncombe and MK Brenner