Immunoglobulin G Subclass Deficiency and Pneumococcal Infection After Allogeneic Bone Marrow Transplantation

By John F. Sheridan, Peter J. Tutschka, Daniel D. Sedmak, and Edward A. Copelan

Serum immunoglobulin (Ig) G subclass levels were measured in a radial immunodiffusion assay in 25 leukemic patients before and after allogeneic bone marrow transplantation. All patients received a conditioning regimen of busulfan and cyclophosphamide followed by infusion of marrow from an HLA-identical sibling. Intravenous infusions of a commercial Ig preparation were administered every 2 weeks until day 120 posttransplant. Nine patients developed pneumococcal infections at 6 months or greater posttransplant. Infection was associated with low levels or the absence of detectable serum IgG2 and IgG4. At the time of infection, 4 of 7 patients evaluated had undetectable IgG2, while 5 of 7 had undetectable levels of IgG4. After infection, none of the 8 patients evaluated had detectable levels of IgG2, and only 2 of 8 had detectable levels of IgG4. In contrast, all 16 patients without pneumococcal infection had IgG2 levels of 102 mg/dL or greater, and IgG4 levels of 20 mg/dL or greater. It appears that IgG2 and IgG4 subclass deficiencies after allogeneic bone marrow transplantation contribute to susceptibility to pneumococcal infection. After pneumococcal infection, IgG2 and IgG4 levels remain low for a prolonged period and patients remain susceptible to infection by encapsulated organisms.

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INFECTION BY encapsulated organisms occurs commonly in individuals with low levels of serum immunoglobulin (Ig) associated with primary and acquired immunodeficiency syndromes.1-3 IgA deficiency is frequently associated with serum subclass deficiency in IgG2,6 or both IgG2 and IgG4.7 While many patients with isolated IgA deficiency do not appear to have an increased risk of infection,8 patients with a concomitant deficiency in the IgG subclasses commonly develop a recurrent pattern of severe upper and lower respiratory tract infections caused mainly by Streptococcus pneumoniae and Hemophilus influenzae.1-4,7,9 The dominant antibody response to capsular polysaccharide antigens occurs predominantly in the IgG2 subclass,10-12 and it probably accounts for the unique susceptibility to infection by encapsulated organisms in these individuals.

Pneumococcal infections occur frequently in long-term survivors of allogeneic bone marrow transplantation (ABMT).13 Typically, these patients have impaired opsonic activity for S pneumoniae and low serum antibody levels for capsular polysaccharide.13 Furthermore, Ig subclass levels have been shown to correlate with the severity of infections in marrow transplant recipients.14 IgG2 and IgG4 antibody levels and antibody responses to capsular polysaccharide antigen of H influenzae have remained abnormally low for as long as 25 months posttransplant.15 In this retrospective study, serum IgG subclasses were measured in 25 long-term survivors of ABMT, nine of whom developed pneumococcal infections. Sixteen patients without infection were studied as controls.

MATERIALS AND METHODS

Patients. Patients were entered on a study protocol that was reviewed and approved by The Ohio State University Institutional Review Board. All patients were conditioned for transplantation with busulfan (16 mg/kg) and cyclophosphamide (120 mg/kg) and received marrow from HLA-identical siblings as treatment for acute myelogenous leukemia, acute lymphocytic leukemia, or chronic myelogenous leukemia. They also received a previously described combination of cyclosporine and methylprednisolone to prevent graft-versus-host disease (GVHD).13 A commercial Ig preparation (Sandoglobulin; Sandoz Pharmaceutical Corp, East Hanover, NJ) was administered intravenously (IV) to each patient at a dose of 500 mg/kg 1 week before transplant and every 2 weeks until day 120 posttransplant. The same Ig preparation was also given orally as a pilot study to test its safety and its effectiveness in prevention of gastrointestinal and systemic infections.16 The daily oral dose was 50 mg/kg in four divided doses starting at day 2 after transplant, and continuing until day 28. Trimethoprim-sulfamethoxazole was given 2 days per week for 6 months posttransplant as prophylactic therapy against Pneumocystis carinii infection.17 Table 1 describes the clinical characteristics of the infected patients. Serum samples were obtained from these individuals before transplantation, before the onset of infection (median time from transplant was 113 days), during infection (median time from transplant was 228 days), and after infection (median time from transplant was 388 days). Serum samples for the uninfected control patients were temporarily matched (days posttransplant) to the infected patients' samples.

Diagnosis of Pneumococcal pneumonia. All individuals presented with fever and demonstrated radiographic evidence of pulmonary infiltration. Blood cultures from at least two different sites were obtained in all individuals. Adequate sputum (in seven patients) or bronchoalveolar lavage fluid (in two patients) was obtained from all nine patients before the administration of antibiotics. Sputum with more than five epithelial cells per high-power field was considered inadequate for meaningful examination. A diagnosis of pneumonia was made only when expectorated sputum or lavage fluid demonstrated predominance of gram-positive diplococci associated with abundant neutrophils in the absence of other predominant flora. In 7 of these 9 patients, S pneumoniae was cultured from sputum, lavage fluid, and/or blood. Cultures were never used as primary diagnostic evidence of pneumococcal pneumonia.

Determination of Ig subclass levels. Serum IgG1, IgG2, IgG3, and IgG4 levels were quantitated by an endpoint radial immunodiffusion assay with an accuracy of ±7% (Miles Scientific, Naperville, IL). All serum samples were either fresh or frozen at −70°C. Each sample was run in duplicate with control sera, and the results were expressed as a percentage of the mean of control sera. A commercial Ig preparation was used to prepare Ig standard curves. A mean of 92 ± 15% (±SD) was obtained for IgG1, 86 ± 15% for IgG2, 90 ± 15% for IgG3, and 76 ± 15% for IgG4 in control sera. The IgG subclass levels were determined by a modification of the radial immunodiffusion method as described by Ouchterlony.18 In this assay, plates were coated with goat antihuman IgG subclass (ImmunoTech, New York). Plates were read after 24 hours at room temperature with a dissecting microscope. Results were expressed as mean diameter of the precipitin arcs in millimeters for each patient at each time point. Results were calculated by subtracting the mean of the normal controls from the mean of the patients for the same time point and then multiplying by 100 to express the result as a percentage. The standard error of the mean was calculated and expressed as a percentage of the mean for each IgG subclass level. A Student’s t test was used to compare means of the infected and normal groups.

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immunodiffusion plate contained a monospecific sheep anti-human antiserum in agarose. Monospecificity was achieved by adsorbing the antiserum against other IgG subclasses. Briefly, serum samples were diluted in sheep serum, and three standards were applied in an immunodiffusion plate containing a monospecific sheep antiserum in agarose. Monospecificity was achieved by adsorbing the antiserum in agarose. After sample application, plates were tightly covered and incubated at room temperature for 24 hours, at which time precipitate diameters were read to 0.1 mm with an immunodiffusion reader (Calibrating Viewer, Transdyne General Corp, Kallestad, Austin, TX). A standard curve for each subclass was generated by plotting the squares of the standard precipitation ring diameter versus their concentration in milligrams per liter on linear graph paper. Patient samples with precipitation rings >9 mm were diluted and repeated. The lower level of sensitivity for each assay was estimated from the standard curve to be: 100 mg/dL for IgG1, 75 mg/dL for IgG2, 12.5 mg/dL for IgG3, and 6 mg/dL for IgG4.

Statistical methods. Linear regression was used to estimate the relationship between time after bone marrow transplantation (BMT) and serum Ig subclass levels. For subclass levels the data are presented as geometric mean titers (GMT) and 95% confidence intervals (lower limit, upper limit). Fisher's exact test was used to detect subclass differences between the infected and noninfected patient groups.

RESULTS

Nine patients developed late (more than 6 months) pneumococcal infections after ABMT (Table 1). All 9 developed pneumonia; 3 were bacteremic, and 3 patients also had sinusitis (patients 2, 3, and 8) or otitis media (patient 2). Four patients developed second, and two patients developed third, episodes of pneumonia with either *S pneumoniae* or *H influenzae* (patients 4, 5, 6, and 8). Five of these 9 patients had chronic GVHD, which was extensive in 3 patients and limited in 2 patients at the time of initial infection. The three patients with extensive chronic GVHD were taking methylprednisolone, azathioprine, and trimethoprim-sulfamethoxazole at the time of infection; however, at least one of the patients frequently skipped doses of trimethoprim-sulfamethoxazole. None of these patients were taking any other antibiotic. None of the nine patients had experienced acute GVHD of grade II or greater. Serum Ig classes were measured at the time of the first pneumococcal infection in seven patients with pneumonia (median time from transplantation was 228 days). IgG levels were normal for 4 of 7 patients, IgM levels were normal for 5 of 7 patients, and IgA levels were low in all 7 patients tested (Table 1).

Serum IgG subclass levels were also measured in the infected and control patients; however, serum samples were not available for every patient for each time period. Serum levels of IgG1 and IgG3 changed only slightly during the course of observation (Table 2). The differences in IgG1 and IgG3 between the infected group and noninfected control group were not statistically significant at any point in time. IgG2 levels declined with time after transplantation in both groups. Among nine noninfected patients who were sampled in each of the four time periods (pre-BMT, and days 96, 204, and 560 after BMT), the regression coefficient of the IgG2 level to the sampling times was -152.9 and was highly significant (*P* = .001). In the infected group, the geometric mean titer for IgG2 was 94 mg/dL before transplantation and declined sharply after infection. While the sample size was too small for a regression analysis, IgG2 titers in the patients with infections were significantly lower than in the uninfected patients (Table 2). On the other hand, serum IgG4 levels showed little variation in the noninfected group but did decline significantly in the infected group (Table 2).

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Infections</th>
<th>Serum Ig (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20/M</td>
<td>AML</td>
<td>Acute</td>
<td>St pn (Pneumonia)</td>
</tr>
<tr>
<td>2</td>
<td>21/M</td>
<td>AML</td>
<td>Chronic</td>
<td>St pn (Pneumonia)</td>
</tr>
<tr>
<td>3</td>
<td>33/M</td>
<td>AML</td>
<td>Chronic</td>
<td>St pn (Pneumonia)</td>
</tr>
<tr>
<td>4</td>
<td>27/F</td>
<td>AML</td>
<td>Chronic</td>
<td>St pn (Pneumonia)</td>
</tr>
<tr>
<td>5</td>
<td>23/F</td>
<td>AML</td>
<td>Chronic</td>
<td>St pn (Pneumonia)</td>
</tr>
<tr>
<td>6</td>
<td>20/F</td>
<td>AML</td>
<td>Chronic</td>
<td>St pn (Pneumonia)</td>
</tr>
<tr>
<td>7</td>
<td>19/F</td>
<td>CML</td>
<td>Chronic</td>
<td>St pn (Pneumonia)</td>
</tr>
<tr>
<td>8</td>
<td>35/M</td>
<td>CML</td>
<td>Chronic</td>
<td>St pn (Pneumonia)</td>
</tr>
<tr>
<td>9</td>
<td>38/F</td>
<td>CML</td>
<td>Chronic</td>
<td>St pn (Pneumonia)</td>
</tr>
</tbody>
</table>

Normal Ig concentrations (mg/dL, range): IgG, 600 to 1,200; IgA, 150 to 350; IgM, 75 to 150.

Abbreviations: CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia; St pn, Streptococcus pneumonia; H influ, Hemophilus influenza; ND, not done.
Table 2. Serum IgG Subclass Levels From Infected and Noninfected BMT Patients

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Serum Sample</th>
<th>Median Time From BMT (range)</th>
<th>N</th>
<th>IgG Subclass Level (mg/dL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IgG1</td>
</tr>
<tr>
<td>Noninfected</td>
<td>Pre-BMT</td>
<td>-2 (-7, 12)</td>
<td>16</td>
<td>997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(852-1,185)</td>
<td></td>
<td>(111-144)</td>
</tr>
<tr>
<td>Noninfected</td>
<td>Sample 1</td>
<td>96 (62, 97)</td>
<td>16</td>
<td>950</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(685-1,318)</td>
<td></td>
<td>(119-151)</td>
</tr>
<tr>
<td>Infected</td>
<td>Before infection</td>
<td>113 (75, 244)</td>
<td>8</td>
<td>1,330</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1,033-1,713)</td>
<td></td>
<td>(59-148)</td>
</tr>
<tr>
<td>Noninfected</td>
<td>Sample 2</td>
<td>204 (106, 368)</td>
<td>14</td>
<td>892</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(650-1,142)</td>
<td></td>
<td>(106-133)</td>
</tr>
<tr>
<td>Infected</td>
<td>During infection</td>
<td>228 (188, 399)</td>
<td>7</td>
<td>1,258</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(630-2,512)</td>
<td></td>
<td>(51-114)</td>
</tr>
<tr>
<td>Noninfected</td>
<td>Sample 3</td>
<td>560 (393, 768)</td>
<td>11</td>
<td>909</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(682-1,212)</td>
<td></td>
<td>(96-107)</td>
</tr>
<tr>
<td>Infected</td>
<td>Postinfection</td>
<td>388 (277, 594)</td>
<td>8</td>
<td>1,103</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(563-2,162)</td>
<td></td>
<td>(30-106)</td>
</tr>
</tbody>
</table>

*Geometric mean titers (95% confidence intervals).
†$P < .01$ by Fisher's Exact test.
‡None of the infected patients had detectable levels of IgG2 antibody.

The regression coefficient for the infected group was $-53.6$ with a $P < .05$. The percent decline of IgG4 between the time intervals of the study was 62% for the "pre-BMT" samples compared with the "before infection" samples, 46% for the "before infection" to "during infection," and 57% for the "during infection" samples to the "postinfection" samples. Thus, patients with pneumococcal infections were those with combined IgG2 and IgG4 deficiency.

There also appeared to be an effect of infection on antibody subclass levels. Infection occurred long after IV Ig therapy was discontinued (median day during infection was 228 after transplant, while IV Ig was discontinued on day 120 after marrow transplant). At the time of infection, 4 of the 7 patients had undetectable IgG2, and 3 patients had low but detectable levels of IgG2 antibody ranging from 120 to 157 mg/dL (Fig 1). None of the seven patients evaluated had detectable IgG2 at a median of 160 days after initial infection. In addition, only 2 of 7 infected patients had detectable IgG4 antibody during and after infection (compared with 15 of 16 noninfected patients that had detectable levels of IgG4).

DISCUSSION

This study confirms the previously described existence of a long-lasting IgG subclass deficiency after ABMT\textsuperscript{14} and shows that these humorally deficient patients are susceptible to recurrent respiratory infections caused by \textit{S pneumoniae}. In prior studies demonstrating the existence IgG subclass deficiency after bone marrow grafting, patients were conditioned for transplantation with a combination of drugs (cyclophosphamide\textsuperscript{14} or busulfan\textsuperscript{29}) and total body irradiation. In this study, total body irradiation was not a part of the conditioning regimen. It is clear that the inclusion of total body radiation in the preparative regimen is not responsible for subclass deficiency after BMT.

More than one mechanism for subclass deficiency may exist in patients who develop bacterial infections. One group (three of the infected patients) had a preexisting deficiency of IgG2 before transplantation, and the deficiency was not corrected in the year after BMT, suggesting that the underlying disorder or previous therapy was responsible for the deficiency. The second group was making detectable levels of IgG2 before BMT and before infection, but ceased to make it after resolution of the infection. In these individuals, bone marrow allografts did not replenish the ability of the host to make Ig of the IgG2 and IgG4 subclasses. Speculation as to the mechanisms operative in the development of this subclass deficiency includes a lack of sufficient T-cell help\textsuperscript{2} for IgG2/IgG4 production as a consequence of the disordered reconstitution of T-cell subsets, and a long-lasting deficit of functional T-cell precursors.\textsuperscript{12}

Two discernible patterns of IgG subclass production were
apparent after transplantation. One group of patients had detectable levels of IgG2 and IgG4, while the second was deficient for IgG2 and IgG4. This latter population appears to be at risk for the development of respiratory infections caused by pneumococcus. This premise is supported by the clinical observation of the second and third episodes of infections by encapsulated bacteria in four patients who remained IgG2- and IgG4-deficient. It is clear that patients with IgG2 and IgG4 deficiencies after marrow transplant are at an increased risk of infection by encapsulated organisms.

Preliminary trials with IV Ig in children with selective IgG2 deficiency have suggested clinical effectiveness. In addition, recent studies have demonstrated that Ig infusions after marrow transplantation reduce the incidence of septicemia and may reduce the frequency of all forms of serious infections. Whether patients who are subclass-deficient after transplantation are specifically benefited has not been clarified, but would be of particular interest. In addition, the clinical resistance of pneumococci to trimethoprim-sulfamethoxazole in our patients and in other studies suggests that this may be an inadequate form of pneumococcal prophylaxis in susceptible individuals.

REFERENCES

Immunoglobulin G subclass deficiency and pneumococcal infection after allogeneic bone marrow transplantation

JF Sheridan, PJ Tutschka, DD Sedmak and EA Copelan