Monoclonal and Oligoclonal Gammopathy After Bone Marrow Transplantation

By Anna J. Mitus, Rosanne Stein, Joel M. Rappeport, Joseph H. Antin, Howard J. Weinstein, Chester A. Alper, and Brian R. Smith

Serial serum protein electrophoreses were performed on 60 patients undergoing allogeneic and syngeneic bone marrow transplantation (BMT). More than 50% of patients (31 of 60) developed transient oligoclonal and monoclonal gammopathies that appeared an average of 84 days posttransplantation (range 27 to 336 days) and persisted an average of 175 days (range 14 to 652 days). Immunofixation analysis revealed 82% of the M components to be of the immunoglobulin G (IgG) type and 18% to be IgM; 56% were κ and 44% were λ. A strong correlation between development of graft versus host disease (GVHD) and appearance of M components was observed (73% incidence in GVHD patients vs 27% in non-GVHD patients, \( P < .0003 \)). Two of the three syngeneic graft recipients also developed monoclonal gammopathies. Evidence of oligoclonal circulating B-cell populations was found in 68% of patients posttransplantation by flow cytometric B-cell excess assay. No correlation of recovery of particular B- or T-lymphocyte subsets and development of M components was seen. The development of transient monoclonal and monoclonal gammopathies after transplantation may be a ubiquitous finding reflecting recapitulation of early B-cell ontogeny.

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MATERIALS AND METHODS

Patient selection. Sixty patients undergoing allogeneic (57) and syngeneic (3) BMT at the Brigham and Women's Hospital and The Children's Hospital between 1976 and 1986 were selected for study. There were 37 males and 23 females aged 1 month to 40 years. The mean age was 18 years. Pretransplantation diagnoses included aplastic anemia (16), acute myelogenous leukemia (14), acute lymphatic leukemia (10), chronic myelocytic leukemia (8), Wiskott Aldrich syndrome (4), severe combined immunodeficiency syndrome (SCIDS) (3), as well as myelofibrosis, Diamond Blackfan syndrome, neuroblastoma, osteopetrosis, and paroxysmal nocturnal hemoglobinuria (1 each). Forty-eight patients received HLA/MLC-matched allografts, nine received grafts from histoincompatible donors, and three received syngeneic grafts. Eighteen of the 60 allografts were T-cell depleted ex vivo either by anti-Leu-1 monoclonal antibody (MoAb) and complement or anti-TiI MoAb plus complement. \(^{15,16}\) This included all HLA/MLC-mismatched donor-recipient pairs as well as nine HLA/MLC-matched patients who qualified for ex vivo T-cell depletion by virtue of recipient age greater than 30 years or sex mismatching of donor and recipient. Myeloablative conditioning regimens were administered to patients transplanted for acute and chronic leukemias, \(^8\) Wiskott Aldrich syndrome, \(^9\) Diamond Blackfan syndrome, \(^9\) osteopetrosis (anithymocyte serum [ATS], total body irradiation [TBI]) and paroxysmal nocturnal hemoglobinuria (PNH) (TBI, ATS, cyclophosphamide). Nonmyeloablative transplant conditioning was administered to patients with aplastic anemia \(^30\) and SCIDS (ATS, cyclophosphamide). The patient transplanted for neuroblastoma received multiple alkylating agents as preparative treatment. Routine GVHD prophylaxis in all patients receiving allografts which were not T-cell depleted ex vivo included a total of only four doses of methotrexate (days 1, 3, 6, and 11) as described \(^31\) and no further immunosuppression. T-cell-depleted patients received either no posttransplantation immunosuppression or the four-dose methotrexate regimen. With the exception of one patient, cyclophosphamide neophylaxis was not routinely administered to any patient. Treatment of acute GVHD consisted of high-dose corticosteroids and/or ATS; chronic GVHD was treated with corticosteroids plus azathioprine. No patient routinely received immunoglobulin replacement.

Serum protein analysis and immunofixation electrophoresis. The 60 patients underwent 1,306 serum protein analyses. Each analysis included a serum protein electrophoresis and immunofixation analysis for immunoglobulin G (IgG), IgM, IgA, and κ and λ light chains. \(^{22}\) IgG, IgA, and IgM were quantified by immunonephelometry. \(^{33}\) Immunoglobulin levels were adjusted for age less than
6 years. Normal levels for patients aged more than 6 years are 600% to 1,500 mg/dL for IgG, 50% to 200 mg/dL for IgM, and 60% to 290 mg/dL for IgA. These analyses were obtained weekly while patients were hospitalized (1 to 2 months posttransplantation) and monthly thereafter. A minimum of 15 studies was available for each patient. All marrow donors also had a single analysis before donation of marrow.

Lymphocyte phenotyping and B-cell clonal excess analysis. Peripheral blood (PB) was obtained after BMT for lymphocyte phenotyping and B-cell clonal excess (α/λ) analysis. PB lymphocytes were isolated by Ficoll-Hypaque gradient separation, divided into 4 to 5 × 10^6 cell aliquots, and incubated with saturating concentrations of phycoerythrin (PE)-conjugated or fluorescein isothiocyanate (FITC)-conjugated MoAbs directed against CD3, CD4, CD5, CD8, CD14, CD16, and CD20 determinants. Correlated dual-parameter data were obtained on a flow cytometer (FACS Analyzer, Becton Dickinson, Mountain View, CA). B-Cell clonal excess was assessed using rabbit F(ab')2 anti-human α and anti-human λ antisera followed by fluorescein isothiocyanate conjugated goat anti-rabbit Ig antisera and analyzed by Kolmogorov-Smirnov statistics.

Statistics. Statistical evaluation was made by microcomputer and Statagraphics software (STSG, Rockville, MD). Data analysis by chi-square, univariate, and multivariate analysis of variance was performed as indicated.

RESULTS

Monoclonal gammopathies. Clinical correlates. Fifty-two percent (31 of 60) of patients studied developed transient oligoclonal gammopathies after BMT, which appeared 84 days posttransplantation on the average (range 27 to 336) and persisted 175 days on the average (range 14 to 652). A representative analysis is shown in Fig 1. A minimum of three bands was visible in all patients, but a major single M component could be defined in 16 cases, and in six instances two M components were identified. Of the 28 M components identified, only IgG and IgM gammopathies were noted, occurring in 82% and 18% of cases, respectively. Of the six clearly identified biclonal M components, in two instances both IgG and IgM M components occurred simultaneously. In the 18 instances in which light chain type was identified, κ and λ light chains occurred with approximately equal frequency (56% vs 44%). No BM donor had evidence of oligoclonal or monoclonal M components.

There was no association of patient age, sex, diagnosis, major histocompatibility complex (MHC) compatibility, or type of GVHD prophylaxis (T-cell depletion v four dose methotrexate) with development of M components as assessed by chi-square analysis (Table 1). Monoclonal gammopathies were observed in 2 of the 3 patients who received syngeneic grafts. These two patients, including one reported previously, had a skin rash clinically and pathologically consistent with GVHD but did not develop intestinal or hepatic signs of this disorder.

There were two clinical correlates for development of monoclonal and oligoclonal immunoglobulin production. First, a strong correlation was detected between development of GVHD and appearance of posttransplantation M components. Of the 33 patients with acute or chronic GVHD, 73% (24 of 33) had transient gammapathies, whereas M compo-

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Table 1. Clinical Correlations of Posttransplantation M Components

<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>No. With M Components</th>
<th>Percentage With M Components</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With</td>
<td>24/33</td>
<td>73</td>
<td>.0003</td>
</tr>
<tr>
<td>Without</td>
<td>7/27</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤14 yr</td>
<td>9/21</td>
<td>43</td>
<td>&gt;.10</td>
</tr>
<tr>
<td>&gt;14 yr</td>
<td>22/39</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>MHC compatibility</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MHC compatible</td>
<td>26/48</td>
<td>54</td>
<td>&gt;.10</td>
</tr>
<tr>
<td>MHC incompatible</td>
<td>3/9</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>T-cell depletion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With T-depletion</td>
<td>9/18</td>
<td>50</td>
<td>&gt;.10</td>
</tr>
<tr>
<td>Without T-depletion</td>
<td>22/42</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>19/38</td>
<td>50</td>
<td>&gt;.10</td>
</tr>
<tr>
<td>F</td>
<td>12/22</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Conditioning regimen</td>
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<tr>
<td>Ablative</td>
<td>26/41</td>
<td>63</td>
<td>-.02†</td>
</tr>
<tr>
<td>Nonablative</td>
<td>5/19</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

*Univariate analysis by chi-square with Yates correction.
†Multivariate analysis of GVHD and conditioning regimen confirmed independent correlation of both GVHD and conditioning regimen at P = .04.
ponents were noted in only 27% of patients without GVHD (9 of 33) \((P = .0003\) by chi-square analysis with Yates correction). Patients in whom de novo chronic GVHD developed, (chronic GVHD not preceded by acute GVHD), had the same high incidence of M components as the group who had acute or acute plus chronic GVHD. Of the 12 patients with de novo chronic GVHD, 67% (8 of 12) had M components. Of the patients with M components, 21 had acute GVHD (7 with grade I, 6 with grade II, 7 with grade III, and 1 with grade IV) and 11 had chronic GVHD. The presence of GVHD did not affect timing or duration of the gammopathies.

A correlation was also noted between the type of conditioning regimen that patients received and development of posttransplantation M components. Specifically, patients receiving “marrow ablative” regimens (defined as conditioning that included either TBI or busulfan) had a higher incidence of M components (63%) than those receiving “nonablative” regimens (26%) \((P = .01\)). There was no correlation of GVHD with conditioning regimen in this study; therefore, as was expected, conditioning regimen and GVHD as compared in a multivariate analysis were independent predictive variables for development of oligoclonal and monoclonal banding on serum electrophoresis.

Two patients in the group of 60 developed posttransplantation lymphoproliferative disease; one patient had an M component posttransplantation that was of the same heavy and light chain class as that of his lymphoma, and the other had no monoclonal gammopathy. Both patients received T-cell–depleted HLA-mismatched allografts. The former developed GVHD posttransplantation that required further immunosuppressive therapy (including MoAbs), and the latter was maintained on cyclosporine prophylaxis posttransplantation.

**Monoclonal gammopathies. Laboratory correlates.** Blood lymphocyte phenotyping was performed on all transplanted patients. Data were analyzed at a date as close to M component appearance as possible. No correlation was evident between the presence of M components and total lymphocyte count or between absolute number or percentage of the following lymphocyte subsets: CD3, CD3CD4, CD3CD8, CD16, CD20CD5, and CD20CD5. No correlation was observed with the CD4/CD8 ratio. Development of M components did, however, relate strongly to quantitative immunoglobulin class levels after transplantation. Those patients without gammopathies demonstrated consistent absolute hypogammaglobulinemia as compared with those with M components. Immunoglobulin levels were compared between the M-component and non-M-component groups at the time of M component appearance and adjusted for age. In the latter group, immunoglobulin levels were obtained at as close to day 84 (average time to M component development) as possible. Of the 31 patients with oligoclonal or monoclonal gammopathies, seven (23%) were hypogammaglobulinemic, whereas 20 of 29 (69%) of patients in whom no M component was demonstrated were hypogammaglobulinemic \((P < .0011\) by one-sided chi-squared analysis). Analysis of mean immunoglobulin levels in the two groups demonstrated the same phenomenon. Although all immunoglobulin classes were diminished in the non M component group, this difference was particularly apparent for IgG (mean 494 v 763 mg/dL; \(P = .04\) by univariate analysis of variance).

**Oligoclonal B cells demonstrated by B-cell clonal excess assay.** Having shown a high frequency of apparent oligoclonal or monoclonal B-cell proliferation after BMT as reflected in secreted immunoglobulin, we determined whether these patients had circulating monoclonal or oligoclonal B-cell populations posttransplantation. B-cell clonal excess was analyzed in 16 patients (eight in the M-component group and eight in the non-M-component group). A clonal population was detected in approximately two thirds of patients tested, with equal frequency in the M-component and non-M-component groups (70% v 67%).

**DISCUSSION**

Transient monoclonal gammapathies after irradiation and marrow reconstitution have been described in animal models. Only scattered reports and two patient series, however, describe the presence of monoclonal gammapathies after human marrow grafting. In the studies involving larger numbers of patients, the incidence of M components was reported to be between 42% and 90%. Vossen et al studied immunodeficiency in 43 children after allogeneic BMT for a variety of disorders. More than 90% demonstrated “homogeneous” immunoglobulins that appeared between 1 and 10 months posttransplantation and persisted for up to 3 to 4 years. The latter were mainly of the IgG and IgM classes, and the six cases examined expressed \(\kappa\) and \(\lambda\) light chain with equal frequency. Hammarstrom et al reported a lesser incidence of monoclonal gammapathies, approximately 42%, and noted a predominance of \(\lambda\) proteins. Our results confirm the common occurrence of M components posttransplantation and agree with the former study in which the two light chain types were found with equal frequency.

What causes monoclonal gammapathy after BMT? Most investigators speculate that the M components represent normal recapitulation of B-cell ontogeny or that they result from a malfunctioning T-cell system and a disturbance of T-B cell cooperation. Support exists for both explanations. Indeed, animal evidence suggests that this phenomenon is in part related to deficient T-cell function in the early posttransplantation period as infusion of additional T cells leads to a reduced incidence of M components in irradiated, reconstituted mice. However, because M components appear transiently when immunoglobulin levels are otherwise normal, and because they occur not only in patients with GVHD but also in patients receiving syngeneic and autologous transplants (Mitus AJ, Stein R, Smith BR, Alper CA, Antin JH: manuscript in preparation), we hypothesize that this phenomenon represents a normal, perhaps ubiquitous element of early B-cell ontogeny. If this is a relatively frequent occurrence after transplantation, how is the clinical association with GVHD explained? Immunologic dysfunction is accentuated in GVHD, further perturbing B-cell and T-cell function and interaction. Thus, occurrence of GVHD...
may render the normal phenomenon of oligoclonal banding more easily detectable.

M components may be associated with malignancies, particularly B-cell lymphoproliferative disorders. However, M components that appear posttransplantation are different from those observed in neoplastic B-cell diseases. In chronic lymphocytic leukemia (CLL), hypogammaglobulinemia and perhaps monochlonal gammopathies (which occur in approximately 5% to 10% of cases) appear to result from primary B-cell dysfunction because addition of normal T cells in vitro does not improve immunoglobulin synthesis.32 An abnormal population of B lymphocytes marked by the CD5 antigen is probably responsible for production of the paraprotein when present in CLL and is phenotypically similar to an unusual population of B lymphocytes found posttransplantation.4 However, unlike CLL, in which these cells are monoclonal, in the few cases that have been studied posttransplantation the CD5+ B lymphocytes are polyclonal. In addition, we found no association between CD5+ B lymphocytes and the appearance of monochlonal gammopathies in our patients. Thus, the appearance of M components posttransplantation probably does not reflect a B-cell neoplastic process. Likewise, development of posttransplantation oligoclonal or monochlonal gammopathy does not appear to herald the later evolution to lymphoproliferative disease. Of the two patients who developed posttransplantation lymphoma, only one demonstrated monochlonal gammopathy. In addition, other risk factors may have precipitated this complication in these patients, including use of HLA/MLC-mismatched donors and posttransplantation immunosuppression.33,34 We cannot interpret the role of Epstein-Barr viral infection in this phenomenon because virtually all patients were antibody positive pretransplantation.

Hematopoietic reconstitution of the BM posttransplantation may occur from a restricted number of clones.35,36 Immune reconstitution probably occurs in a clonal or oligoclonal manner as well, and the oligoclonal banding detected may represent, as suggested by Radl, "unequal development of several rapidly differentiated clones of immunoglobulin-producing cells."15

The donor versus recipient origin of the monoclonal protein found posttransplantation remains uncertain. Although most evidence indicates a conversion of blood-borne cells and serum immunoglobulins to donor type by day 100 in most posttransplant recipients,35,36 other studies have shown chimeric lymphocyte populations 6 months or more after transplantation.38-40 Therefore, the M components noted in our patients may be of host rather than of donor origin. That M components occurred more frequently in patients who received ablative conditioning regimens in this study suggests that at least some are donor derived; mixed chimerism is less common in patients receiving such preparative conditioning. Previous studies attempting to examine the donor-versus-host origin of M components by using Gm typing proved unsuccessful.14

Evidence for monoclonal and oligoclonal B-cell proliferation within the first year after BMT can be found in most BMT recipients. Although this occurs most frequently in patients with GVHD, it does not appear to be associated with any long-term adverse consequences of transplantation.

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