Immunoglobulin E Levels Following Allogeneic, Autologous, and Syngeneic Bone Marrow Transplantation: An Indirect Association Between Hyperproduction and Acute Graft-v-Host Disease in Allogeneic BMT

By Judith Heyd, Albert D. Donnernberg, William H. Burns, Rein Saral, and George W. Santos

Markedly elevated serum IgE levels have been noted following allogeneic bone marrow transplantation (BMT) and have been correlated with graft-v-host disease (GVHD) in several studies. To investigate this phenomenon, we measured serum IgE levels in 387 allogeneic, 143 autologous, and 21 syngeneic BMT recipients before and at intervals after BMT. As a population, allogeneic BMT recipients displayed a biphasic elevation in IgE levels, with peak levels occurring either early (days 15 to 19) or late (days 80 to 89) posttransplant. Only in individuals in whom peak levels occurred early did IgE level correlate with liver disease, histological changes, and overall clinical stage of GVHD. The association of IgE elevation and GVHD does not appear to be direct since recipients of syngeneic (monozygotic twin) grafts had the highest incidence of IgE hyperresponsiveness as well as the highest absolute IgE levels. Similarly, 22 recipients of autologous marrow not treated with 4-hydroperoxycyclophosphamide had elevated IgE levels comparable to those seen in allogeneic graft recipients. We hypothesize that augmented IgE synthesis and its subsequent resolution is the natural consequence of immune reconstitution in the presence of potentially reaginic agents such as antibiotics and infectious agents. As such, IgE hyperresponsiveness in syngeneic graft recipients may reflect the maturational sequence of IgE regulatory elements in the absence of interference by GVHD, GVHD therapy, or minor histocompatibility disparities. The cell populations required for IgE response (T cells, B cells, and antigen-presenting cells) may be reconstituted in advance of the regulatory elements that limit IgE production in healthy subjects. Although this temporal relationship does not appear to hold in allogeneic BMT, the balance between positive and negative factors, which determines the rates of IgE synthesis and catabolism, may be altered by GVHD, infection, and liver dysfunction acting alone or in combination.

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MATERIALS AND METHODS

Patient population. The patients in this study received transplants at the Johns Hopkins Oncology Center between March 1977 and April 1986. A total of 551 patients with evaluable IgE data were included in this series. Of these patients, 387 received allogeneic, 143 received autologous, and 21 received syngeneic transplants. All available immunoglobulin data from day -21 to day 100 after BMT were evaluated. In most patients, immunoglobulin determinations were performed on a weekly basis. Pretransplant diagnoses are summarized in Table 1 by BMT type. A subset of this patient group was used for the purpose of analysis of the relationship of immunoglobulin levels and GVHD. This group included 216 allogeneic transplant patients for whom both IgE and GVHD data were available.

Immunoglobulin. Determinations of immunoglobulin content were performed by radioimmunooassay (IgE) and rate nephelometry (IgG) by the Johns Hopkins Department of Laboratory Medicine.

GVHD. GVHD was evaluated on a weekly basis as previously described. Briefly, four categories of GVHD were scored on a scale of 0 (no evidence of disease) to 4 (severe disease). The categories included (a) histological grading of skin biopsy material, (b) liver GVHD as determined by laboratory values and histology when available, (c) gastrointestinal GVHD as determined primarily by stool output, and (d) overall clinical stage, which takes into account all of the aforementioned parameters.

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IgE Levels in BMT

**Table 1. Patient Characteristics**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Allogeneic*</th>
<th>Autologous</th>
<th>Syngeneic</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>87</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ALL</td>
<td>104</td>
<td>46</td>
<td>7</td>
</tr>
<tr>
<td>ANLL</td>
<td>125</td>
<td>39</td>
<td>6</td>
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<td>CML</td>
<td>47</td>
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<tr>
<td>HD</td>
<td>7</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>NHL</td>
<td>4</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>13</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>387</td>
<td>143</td>
<td>21</td>
</tr>
</tbody>
</table>

*Number of patients studied.

Abbreviations: AA, aplastic anemia; ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia; CML, chronic myelocytic leukemia; HD, Hodgkin’s disease; NHL, non-Hodgkin’s lymphoma.

**RESULTS**

*Serum IgE and IgG levels following BMT.* Serum IgE and IgG levels, by BMT type, are shown as a function of time post-BMT (Fig 1). A total of 3,466 IgE determinations and 3,319 IgG determinations were obtained for 551 patients. Two-way ANOVA was performed on immunoglobulin levels by BMT type and time post-BMT. Pretransplant IgE levels were indistinguishable between recipients of allogeneic, autologous, and syngeneic transplants. Geometric means (lower and upper 95% confidence intervals) were 47.5 (39.0, 57.8), 47.1 (34.3, 64.9), and 55.1 (26.2, 115.7) ng/mL for 299 allogeneic, 104 autologous, and 21 syngeneic BMT recipients for whom pretransplant data were available (Fig 1A). Both allogeneic and syngeneic BMT recipients evidenced significant elevations in serum IgE compared with pretransplant levels (P < .001), with maximal levels occurring 3 weeks post-BMT. Although changes in serum IgE levels in these two groups paralleled each other, the magnitude was significantly greater in recipients of syngeneic grafts (P < .001). Serum IgE levels did not change significantly from pretransplant levels in autologous BMT recipients.

Pretransplant IgG levels (Fig 1B) were also indistinguishable between BMT types. Arithmetic means (lower and upper 95% confidence intervals) were 716 (672, 759), 750 (662, 838), and 765 (612, 918) for 227 allogeneic, 65 autologous, and 17 syngeneic BMT recipients, respectively. In all three BMT groups, IgG levels declined significantly from their initial levels and reached a nadir between weeks 0 and 1 (days 0 and 13) post-BMT. In autologous recipients, IgG levels remained depressed through week 15, whereas they approached pretransplant levels by weeks 4 to 5 in autologous and syngeneic BMT recipients. Thus, IgG levels in the allogeneic group were significantly depressed compared with both autologous and syngeneic BMT groups.
(P < .001). IgG recovery did not differ significantly between recipients of autologous and syngeneic grafts.

**Maximal IgE levels post-BMT.** For detailed analyses, a subset of the aforementioned data was chosen on the basis of availability of serial IgE data and GVHD data (allogeneic BMT patients). Two hundred sixteen allogeneic, 116 autologous, and 20 syngeneic transplant patients were studied. Maximum IgE levels, the days on which these occurred, and the maximal changes in IgE relative to pretransplant levels (ratio of maximum to pretransplant IgE levels) were recorded and compared by ANOVA between BMT types. The data (Table 2) indicate that following BMT all groups differ from each other with respect to maximal IgE levels (absolute level and increase relative to pretransplant levels, P < .004 in all comparisons). When using a cut point of 775 ng/mL (the upper 75th percentile of the allogeneic data set) as an arbitrary level for greatly elevated IgE, 51 of 216 allogeneic and 12 of 20 syngeneic recipients met this criterion (chi-square, 14.41; P < .001). Only four of 116 autologous recipients fell into this category. Interestingly, when recipients of autologous grafts were grouped by the method used for in vitro tumor purging (4-hydroperoxycyclophosphamide [4-HC], n = 45; no treatment or monoclonal antibody treatment, n = 22), maximal IgE levels in the group that did not receive 4-HC were indistinguishable from those of the allogeneic group. The day on which individuals experienced their maximal IgE level was bimodally distributed in the allogeneic population, with a major mode between days 15 and 19 (n = 45) and a minor mode between days 80 and 89 (n = 10). The distributions appeared to be unimodal in the autologous and syngeneic BMT groups, but follow-up was limited to approximately 60 days. No difference was detected in the median day of maximal IgE levels between the groups (P > .05 in all cases).

**Correlation with GVHD.** To determine whether IgE elevation correlates with GVHD in the allogeneic transplant group, patients were grouped into three IgE groups (low, intermediate, and high) and two GVHD groups (none to mild v moderate to severe). Cut points for IgE groups were at the 25th and 75th percentiles of the allogeneic data set (92 and 775 ng/mL). To determine whether the time at which the maximal IgE level is attained influences such correlations, the data were divided according to whether peak levels were attained before (n = 168) or after (n = 48) day 40, a time after which the risk for developing acute GVHD is minimal. Two-way tables comparing the frequencies of the various IgE group/peak time group/GVHD group combinations were constructed and tested for statistically significant associations by Pearson’s chi-square analysis. The results (Table 3) indicate that, in patients experiencing peak IgE levels before day 41, overall clinical stage, histological grade, and liver stage GVHD parameters were highly correlated with maximal IgE levels attained after BMT. Gastrointestinal GVHD was weakly correlated with maximal IgE level (P = .08). No associations were seen between GVHD parameters and IgE in patients experiencing maximal IgE levels after day 40. Despite this finding, regression analysis failed to detect a correlation between the day of maximal IgE content and the day of maximal GVHD (all GVHD parameters). The ability to detect such a correlation may have been limited by the frequency of GVHD and immunoglobulin data collection (approximately weekly).

To further investigate the correlation of liver GVHD and IgE, LFT values (SGOT, SGPT, SALK, TBLI) obtained on the day that patients reached maximal serum IgE levels were correlated with those maximal values expressed both as absolute levels (ng/mL) and as ratios relative to pretransplant values. Surprisingly, when absolute maximal IgE levels were evaluated, no correlations were detected between IgE and any of the liver parameters. However, assessment of maximal IgE level, expressed as a ratio of the pretransplant value, revealed strong statistical associations between this parameter and SGOT, SGPT, and SALK and a modest correlation with TBLI (Table 4). As with the GVHD data, these correlations were limited to individuals experiencing maximal IgE levels prior to day 41. Despite these strong correlations, analysis of syngeneic transplant data revealed no association between IgE and LFT values. In fact, only three of 21 syngeneic BMT recipients had even moderately elevated LFT results.

Other parameters that were evaluated by chi-square anal-

### Table 2. Maximal IgE Levels Following BMT

<table>
<thead>
<tr>
<th>BMT Type</th>
<th>Maximal IgE (ng/mL)*</th>
<th>Day of Maximum IgE†</th>
<th>Maximal Change in IgE‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allogeneic (n = 216)</td>
<td>276 (221, 343)</td>
<td>28 (25, 31)</td>
<td>5.3 (4.0, 7.0)</td>
</tr>
<tr>
<td>Syngeneic (n = 20)</td>
<td>1,018 (407, 2,545)</td>
<td>25 (19, 31)</td>
<td>18.4 (13.3, 41.0)</td>
</tr>
<tr>
<td>Autologous (n = 116)</td>
<td>77 (60, 99)</td>
<td>21 (18, 24)</td>
<td>2.2 (1.6, 2.8)</td>
</tr>
<tr>
<td>4-HC purged (n = 94)</td>
<td>58 (45, 75)</td>
<td>21 (18, 24)</td>
<td>2.0 (1.5, 2.8)</td>
</tr>
<tr>
<td>Unpurged (n = 22)</td>
<td>268 (168, 427)</td>
<td>24 (18, 31)</td>
<td>2.5 (1.4, 4.7)</td>
</tr>
</tbody>
</table>

*Geometric means, lower and upper 95% confidence intervals (parentheses).
†Days after BMT to highest measured IgE level. Arithmetic means, lower and upper 95% confidence intervals (parentheses).
‡Ratio of maximal and pretransplant IgE levels.

### Table 3. Correlation of Maximal IgE Level With Parameters of GVHD by Time of Maximal IgE Level

<table>
<thead>
<tr>
<th>GVHD Parameter</th>
<th>Maximal IgE ≤ Day 40 (n = 168)</th>
<th>Maximal IgE &gt; Day 40 (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-Square*</td>
<td>P Value</td>
<td>Chi-Square*</td>
</tr>
<tr>
<td>Overall clinical stage</td>
<td>8.42</td>
<td>.015</td>
</tr>
<tr>
<td>Histological grade</td>
<td>8.57</td>
<td>.014</td>
</tr>
<tr>
<td>Liver stage</td>
<td>17.49</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Gastrointestinal stage</td>
<td>5.15</td>
<td>.076</td>
</tr>
</tbody>
</table>

*Pearson chi-square statistic (2 degrees of freedom): IgE data were grouped into three categories (low, intermediate, and high); GVHD data were grouped into two categories (none to mild, moderate to severe).
†Probability not reported due to sparseness of fitted cells (frequency, <5 in more than two cells).
The effect was abrogated when marrows were treated in vitro and evened higher levels at a later time. Serum IgE values were also elevated in recipients of autologous grafts, but this did not reflect differences in the immune reconstitution of allograft recipients.

Serum IgE values were grouped into three categories (low, intermediate, and high); liver data were split into two categories. The correlation between an increase in LFT values and elevated IgE levels was also correlated with elevation of LET values in this group, thus reflecting the effects of liver GVHD plus other causes of liver toxicity. In syngeneic recipients serum IgE levels were found to be significantly elevated in the allogeneic and syngeneic BMT recipients, in some cases far above those seen in atopic individuals, which confirms previous observations.

In our allogeneic population, IgE levels followed a bimodal pattern, peaking between days 15 and 19 in some individuals and between 80 and 89 in others. In allogeneic patients experiencing peak IgE levels early after BMT, IgE elevation correlated strongly with GVHD, particularly GVHD of the liver. Elevation of IgE levels was also correlated with elevation of LFT values in this group, thus reflecting the effects of liver GVHD plus other causes of liver toxicity. In syngeneic recipients serum IgE rose to significantly higher levels than in the allogeneic BMT group. These results are consistent with those of Ringden et al who reported very high serum IgE levels in one syngeneic BMT recipient.4 Like the majority of allogeneic patients, our syngeneic recipients experienced maximal IgE levels between days 19 and 31. Due to shorter follow-up, it is not known whether some of these individuals would have experienced even higher levels at a later time. Serum IgE values were also elevated in recipients of autologous grafts, but this effect was abrogated when marrows were treated in vitro with 4-HC prior to freezing.

The patterns observed in the different BMT populations most likely reflect differences in the tempo of regeneration of cells involved in IgE production and regulation. IgE synthesis is highly regulated and has been shown to be under the control of both positive and negative regulatory populations.11 In recipients of syngeneic and unpurged autologous grafts, transient IgE hyperproduction is common. This suggests that the synthetic machinery required to produce IgE is in place early, whereas the regulatory elements that keep IgE levels many logs below that of IgG in normal individuals12 are recovered somewhat later. The absence of IgE hyperproduction in recipients of 4-HC–treated allogeneic marrow indicates that one or more of the components responsible for IgE hyperproduction is graft derived. However, the finding that IgE is virtually absent in allogeneic transplant recipients who do not suffer acute GVHD or liver dysfunction points to a fundamental difference in the immune reconstitution of allograft recipients.

A mechanism similar to that involved in the “allogeneic effect” phenomenon has been proposed by Ringden et al to account for GVHD-associated helper effects.5 This would classically involve the collaboration of donor T cells with residual recipient B cells. In experimental animal models of GVHD such cooperative effects can be demonstrated, but the window of time during which they occur is brief and early (6 days posttransplant).13 The alternative but not mutually exclusive possibility that GVHD-associated IgE hyperproduction results from an abrogation of suppression implies that allogeneic patients normally evolve a mechanism (specific or nonspecific) capable of preventing IgE hyperresponsiveness. Disruption of this mechanism by GVHD, with or without heightened helper response, would predictably result in increased IgE synthesis. The sequential development of nonspecific and specific suppressor responses following allogeneic BMT has been described in alloantigen-specific systems. Such mechanisms are believed to play a role in prevention and/or recovery from GVHD.14

The absence of specific immune regulatory mechanisms may also contribute to the chronically elevated IgE levels seen in association with certain congenital immunodeficiency syndromes15 and neoplasms.16,17

In our allogeneic data set the significance of liver disease per se remains problematic since liver disease of multiple etiologies including alcoholic cirrhosis and viral hepatitis is associated with elevated IgE levels.18,19 Ideally, liver GVHD would be diagnosed histologically rather than biochemically and could therefore be distinguished from other toxicities such as venoocclusive disease and hepatitis. In reality, confirmatory biopsy material was available in only 16 of 75 cases of biochemically defined liver GVHD. An additional eight biopsies were performed on individuals who did not fit the biochemical criteria.7 Concordance in this series was 79% (19/24). Misclassifications were evenly distributed between false positives (n = 2) and false negatives (n = 3). When individual biochemical indicators of liver dysfunction were evaluated, only the allogeneic BMT group evidenced a correlation between an increase in LFT values and elevated IgE levels. In the rare autologous and syngeneic recipients who had GVHD (one of each group), no increases in IgE levels or LFT values were detected. Therefore, liver disease may contribute to the elevation of IgE levels or enhance its magnitude once established but cannot, in itself, account for it.

Additionally, we examined the allogeneic data set for the effect of other factors reported to influence levels of serum IgE in humans or in rodents, namely, age,8 treatment with cyclosporine,20 and irradiation.21 None of these factors had a
significant effect on the maximal IgE levels observed in allogeneic recipients.

Finally, the fact that IgE hyperresponsiveness is transient in recipients of syngeneic, allogeneic, and unpurged autologous grafts suggests that either the stimuli provoking this aberrant response are removed or, more probably, that the physiological mechanisms of immunoregulatory control are restored. If the latter interpretation is correct, future studies directed at the mechanism of recovery from IgE hyperresponsiveness in BMT may be of relevance to atopic populations as well. The ability to detect and monitor the activity of human IgE regulatory factors will facilitate this endeavor.22

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