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

By Judith Heyd, Albert D. Donnenberg, 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-vs-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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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 I 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.

Immune regulation. Determinations of immunoglobulin content were performed by radioimmunossay (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.

Patient data were stored in a microcomputer-based data base (PDBase, IOTC Inc, Laramie, WY) running on a Corvus Concept (San Jose, CA) computer. Statistical analysis was performed by using SYSTAT (Systat Inc, Evanston, IL) running on an IBM personal computer. As in the general population, the distribution of IgE values in our population was skewed toward the right. A log transformation of IgE levels normalized this distribution and was therefore used for the computation of means and in analysis of variance (ANOVA). All other parameters (including IgG levels) were approximately normally distributed. For chi-square analysis, GVHD data were divided into two groups, no or mild GVHD and moderate or severe GVHD, corresponding to numerical scores of 0 to 1 and 2 to 4, respectively. Continuous variables such as immunoglobu-
IgE LEVELS IN BMT

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Allogeneic*</th>
<th>Autologous</th>
<th>Syngeneic</th>
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<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>
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</tr>
<tr>
<td>ANLL</td>
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<td>6</td>
</tr>
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<td>2</td>
</tr>
<tr>
<td>HD</td>
<td>7</td>
<td>6</td>
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</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>

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.

*Number of patients studied.

Platelet levels and liver function test (LFT) results were grouped into categories. Immunoglobulin levels were divided into three categories corresponding to the hinge points (25th and 75th percentiles) of the allogeneic BMT data set. LFT results were divided into two categories by using the cut points used to define GVHD of the liver. These were SGOT, ≥ 150; SGPT, ≥ 150; serum alkaline phosphatase (SALK), ≥ 120; total bilirubin (TBLI), ≥ 3.5.

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 allogeneic 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.

Fig 1. Mean serum IgE levels (A) and IgG levels (B) by BMT type as a function of time post-BMT. IgE levels are expressed as the base 10 logarithm of IgE concentration in nanograms per milliliter.
detected in the median day of maximal IgG bevels between = days 1 5 and 19 (n experienced their maximal IgE level was bimodally distributed in the albogeneic group, with a major mode between days 80 and a minor mode between days 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 analysis 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).

**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 or 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).

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Other parameters that were evaluated by chi-square analysis**
effect was abrogated when marrows were treated in vitro enced even higher levels at a later time. Serum IgE values suggest that the synthetic machinery required to produce IgE is is highly regulated and has been shown to be under the control of both positive and negative regulatory populations. 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 individuals are recovered somewhat later. The absence of IgE hyperproduction in recipients of 4-HC-treated autologous 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. 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). 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. The absence of specific immune regulatory mechanisms may also contribute to the chronically elevated IgE levels seen in association with certain congenital immunodeficiency syndromes and neoplasms.

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. 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. Concordance in this series was 79% (19/24). Misclassifications were evenly distributed between false positives (n = 5) and false negatives (n = 4). 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, treatment with cyclosporine, and irradiation. None of these factors had a

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### Table 4. Correlation of Amount of Increase in Maximal IgE Levels With Parameters of Liver Function by Time of Maximal IgE Level

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Maximal IgE ≤ Day 40 (n = 168)</th>
<th>Maximal IgE &gt; Day 40 (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi-Square*</td>
<td>P Value</td>
</tr>
<tr>
<td>SALK</td>
<td>12.18</td>
<td>.002</td>
</tr>
<tr>
<td>SGOT</td>
<td>6.83</td>
<td>.033</td>
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<tr>
<td>SGPT</td>
<td>7.60</td>
<td>.022</td>
</tr>
<tr>
<td>TBLI</td>
<td>5.68</td>
<td>.058</td>
</tr>
</tbody>
</table>

*Pearson chi-square statistic (2 degrees of freedom): IgE data were grouped into three categories (low, intermediate, and high); liver data were grouped into two categories (see the text).
†Probability not reported due to sparseness of fitted cells (frequency, <5 in more than two cells).
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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REFERENCES

1. Geha RS, Rappaport JM, Twarog F, Parkman R: Serum IgE levels following allogeneic bone marrow transplantation in man. Monogr Allergy 14:81, 1979
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