Markedly Elevated Serum IgE Levels Following Allogeneic and Syngeneic Bone Marrow Transplantation

By Olle Ringdén, Ulla Persson, S.G.O. Johansson, Henryk Wilczek, Gösta Gahrton, Carl-Gustav Groth, Göran Lundgren, Berit Lönnqvist, and Erna Möller

Serum IgE levels were studied in 25 bone marrow transplant recipients (in 12 patients twice weekly and in 13 patients, at random). A 2-748-fold increase in serum IgE was recorded in 20 of the 25 patients after transplantation. The highest IgE value observed being 8,000 kU/liter. The IgE elevation appeared concomitantly with acute graft-versus-host disease (GVHD) in 14 patients. Both events occurred on day 24 ± 2 (mean ± SE). When the acute GVHD was diagnosed, there was a significant increase in serum IgE as compared to the first posttransplantation value. In one patient in whom GVHD recurred, a second IgE peak was seen, and in another patient with flaring GVHD, IgE levels increased on several occasions. In 6 patients without clinical signs of GVHD, a rise in IgE occurred on day 35 ± 12. One of these patients was grafted with marrow from her identical twin. The rise in IgE did not correlate with an elevated proportion of eosinophil granulocytes. In the majority of the patients, no correspondent increases in serum IgG, IgA, or IgM were seen during the period with increased IgE after transplantation.

GRAFT-VERSUS-HOST disease (GVHD) represents one of the major obstacles to successful allogeneic bone marrow transplantation. The acute form, which appears during the first 2 mo after transplantation, develops in approximately 70% of the recipients of marrow from HLA-matched siblings. The disease mainly affects the skin, gut, and liver and is diagnosed on the basis of findings in tissue biopsies. Patients with GVHD have an increased mortality in infections, which may be caused by a slower immunologic recovery compared to patients without GVHD. However, more than 6 mo after transplantation, patients with GVHD were reported to have significantly higher than normal IgG levels. Furthermore, in vitro stimulation of lymphocytes with polyclonal B-cell activators resulted in a transient markedly increased polyclonal IgG secretion in patients with acute GVHD. Recently, Geha et al. found an elevation in serum IgE levels following allogeneic bone marrow transplantation, and in half of the patients, the rise accompanied acute GVHD. In patients treated with rabbit antithymocyte serum, the rise in IgE was paralleled by a strongly positive radioallergosorbent (RAST) assay to rabbit serum protein. We have studied serum IgE levels in 25 bone marrow recipients not given prophylactic antithymocyte serum and have found elevated IgE levels in most of them.

MATERIALS AND METHODS

Patients

Twenty-five marrow transplant recipients were included in the analysis. Three patients had aplastic anemia (AA), 11 had acute nonlymphoblastic leukemia (ANL) in first remission, 1 had ANL in second remission, 1 ALL patient was in his third remission, and 2 patients had chronic myelogenous leukemia (CML) in the chronic phase. Table I lists age, sex, diagnosis, remission status before transplantation, and survival. Also listed are severity of GVHD seen in 17 patients, the day when GVHD appeared, and some serum IgE data.

Treatment

All donors were siblings. Patient L30 was transplanted with marrow from an identical twin. Patient L16 received marrow from his HLA-A, B, C-identical but D-nonidentical sister. HLA-DR typing showed that the recipient had DR2, which the donor did not have, and that mixed lymphocyte cultures were mutually reactive. All other recipient/donor pairs were HLA-identical. Treatment has previously been described in detail. In brief, patients with AA were conditioned with 50 mg/kg cyclophosphamide on each of 4 successive days. Patients with leukemia received 60 mg/kg cyclophosphamide on each of 2 successive days, followed by 10 Gy delivered by a linear accelerator. The lungs were shielded to receive no more than 9 Gy. All patients received several transfusions during the first weeks after transplantation. The blood products were irradiated with 15 Gy.

Patient L33 received cyclosporin-A, 12 mg/kg/day orally, for prophylaxis of GVHD. The other patients were given intermittent methotrexate for the first 100 days after marrow-grafting, using the Seattle protocol. If clinical signs of acute GVHD appeared, prednisolone was introduced in doses of 2 mg/kg/day during the first week; then the dose was tapered. If GVHD recurred in an acute form, some patients also received i.v. methylprednisolone. One of the patients (L22) with severe GVHD was also treated with rabbit antithymocyte globulin (RATG), thoracic duct drainage, and azathioprine (see Fig. 2).

Determination of Serum Immunoglobulin Levels

Serum IgE levels were determined by a sandwich-type radioimmunoassay (RIA), using the commercially available reagents, Phad-
Table 1. Clinical Features and IgE Data

<table>
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<th>Patient Number</th>
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<th>Grade†</th>
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<th>Increase Observed</th>
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<th>Max Increase</th>
<th>Fold Increase</th>
<th>Survival (mo)</th>
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<td>AA</td>
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<td>II-Chronic</td>
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<tr>
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<tr>
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<td>10</td>
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<td>6</td>
<td>150</td>
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*AA, aplastic anemia; AML, acute myelogenous leukemia; EL, erythroleukemia; APML, acute promyelocytic leukemia; AMOL, acute monocytic leukemia; ALL, acute lymphoblastic leukemia; AMML, acute myelomonocytic leukemia; CML, chronic myelogenous leukemia in chronic phase; T-ALL, T-cell leukemia.
†Using Seattle criteria.
‡Transplantation, mean value before increase.
§Patients A4 through L16 were tested at random (from 2 to 11 times); patients L21–L33 were tested twice weekly (see Materials and Methods).
‖Remission status.
§§Not tested during acute GVHD.
**Twin donor.

RESULTS

Increased Serum IgE After Transplantation

Before transplantation, the patients had mean ± SE serum IgE levels of 10 ± 2 kU/liter, compared to 28 ± 9 in the donors. After bone marrow transplantation, serum IgE levels increased 2–748-fold (103 ± 47-fold) compared to baseline levels after transplantation. Elevations in serum IgE were seen in 20 of the patients. This increase was noted from day 13 to day 34 after transplantation in 11 patients who were followed twice weekly. In these patients, the IgE elevation lasted between 7 and 52 days (mean ± 5).

IgE Elevation Occurs Concomitantly With Acute GVHD

In 14 patients with acute GVHD, the increase in serum IgE appeared concomitantly with acute GVHD. Both events occurred on day 24 ± 2 after transplantation (Table 1). Signs of acute GVHD appeared from day 10 to day 34 after transplantation. In these patients, the IgE elevation was seen from day 12 to day 32. In patients with acute GVHD, the first IgE value determined after transplantation was compared to the IgE value taken closest to diagnosis of acute GVHD in 10 patients followed frequently enough to allow analysis. The increase in IgE occurring at the diagnosis of acute GVHD was significant (p < 0.01 using Wilcoxon matched-pairs signed-rank test and p = 2.6 × 10⁻⁸ using paired t test of log IgE values). A typical
course is shown in Fig. 1. A 28-yr-old female with ANL (L21) developed a skin rash on day 17 after transplantation. A skin biopsy on day 18 showed acute GVHD, and prednisolone treatment was instituted on day 19. The rash disappeared on day 24. On day 20 there was a fourfold increase in serum IgE levels to 98 kU/liter.

In patient L22, a second peak of elevated IgE values occurred when the acute GVHD reappeared and became worse. The clinical course of this patient, a 23-yr-old male with acute monocytic leukemia, is shown in Fig. 2. Because of the rise in bilirubin, the appearance of diarrhea, and skin rash, prednisolone was instituted on day 34. Previously taken liver and skin biopsies showed no characteristic findings. The skin rash disappeared, but reappeared on day 41 when the skin biopsy showed signs of GVHD. The skin condition became worse and RATG was added on day 52 and given for 6 days. The skin condition improved and remained stable for some time, but the skin
Elevated IgE After Marrow Transplantation

Serum IgE levels increased at several occasions after transplantation. Flare of acute GVHD is evident by rash and treatment with methylprednisolone (indicated with arrows). In this patient, some IgE values below 2.5 kU/liter were not further analyzed and are indicated as 2.5 kU/liter.

GVHD flared up again on day 89. Thoracic duct drainage was started; i.v. methylprednisolone, RATG, and azathioprine were given. In spite of all immunosuppressive treatment, the skin rash became worse, and bilirubin and liver enzymes increased. The patient died of bronchopneumonia 116 days after transplantation. The two peaks of IgE appeared concomitantly with active acute GVHD. During the second IgE peak, the patient's serum contained small amounts of anti-rabbit IgG antibodies, which did not interfere with the measurement of IgE.

Patient L28 had persistent GVHD of the skin and gut, and IgE levels were therefore followed for a prolonged period (Fig. 3). Prednisolone was instituted on day 23. His serum IgE levels increased sixfold on day 17, with a maximal 14-fold elevation on day 28. His GVHD was stable for some weeks, but then became worse. Methylprednisolone was given i.v. for 3 days from day 60. This patient had a fivefold IgE rise on day 63, compared to previous IgE levels. The patient continued to have gut problems, and from day 88, he was treated with cyclosporin-A. His IgE levels were unstable, and on 3 occasions elevated IgE levels had appeared when GVHD flared up. He died 7 mo after transplantation of bleeding associated with severe GVHD.

Elevated IgE in Patients Without GVHD

The level of IgE also increased in 6 patients without clinical signs of acute GVHD. The IgE increased 88 ± 82-fold in these patients. In 14 patients who developed acute GVHD, the IgE increased 109 ± 58-fold (Table 1). In the patients without GVHD, elevation of IgE values was observed on day 35 ± 12 (range days 16–91). In patient L30 with CML, who was a recipient of a twin-transplant, the increase in serum IgE started on day 16. Her maximum IgE value was 8,000 kU/liter, determined on day 30.

No Correlation Between Eosinophil Counts or "Take" and IgE Levels

After transplantation, an increased proportion of eosinophils in the white cell differential count (>4%) was seen on 10 occasions in 9 patients. On 5 occasions, this increase occurred during acute GVHD and on 5 occasions it did not. In patient L30 with an identical twin donor, increased eosinophil counts appeared concomitantly with elevated IgE levels. In the other 19 patients with increased IgE levels, no correlation between a rise in the proportion of eosinophil granulocytes and the elevation of IgE was seen. The day of engraftment or "take," defined as a stable leukocyte level above 0.2 × 10⁹ cells/liter after the posttransplantation nadir, was not correlated with IgE elevation.

IgG, IgA, and IgM Values After Transplantation

In most patients, an increase (>100%) in one or more of these immunoglobulin (Ig) classes occurred during the first weeks after transplantation. These increases sometimes occurred during the IgE elevation, but in most patients, the increase in other Ig
classes was less than 100%. Furthermore, with very few exceptions, the values did not exceed the upper limit (+2 SD) of the normal range. In one patient with GVHD, a gradual decrease in IgA and IgM was observed. Thus, the changes in IgG, IgA, and IgM did not form any consistent pattern, and there was no obvious correlation between the changes observed in the various Ig classes.

**DISCUSSION**

In most patients studied following bone marrow transplantation, we found a marked increase in IgE levels, as previously reported by Geha et al. They reported that a significant part of the IgE response was due to specific antibodies to rabbit serum proteins, since patients treated with rabbit antithymocyte serum developed a positive RAST to rabbit serum. However, the anti-IgE used in the RAST test is of rabbit origin and we believe that the presence of antibodies directed against rabbit gammaglobulin may give false positive RAST test to rabbit serum. One of our patients (L22) was treated with RATG and developed a small amount of IgG antibodies directed against rabbit gammaglobulin. However, the presence of these antibodies did not significantly affect the measurement of total IgE.

It has not yet been determined whether the IgE-producing B cells are of host or donor origin. A previous study of IgG allotypes in bone marrow transplant recipients showed that all immunoglobulins were of donor origin, from 4 to 12 mo after transplantation. The IgE increase generally appeared during the first month. Since no allotypes have yet been discovered for IgE in humans, no conclusion can be drawn at present.

Markedly increased IgE levels have been reported in atopy, parasitic infections, and various diseases associated with deficient T-cell function. Bone marrow transplant recipients are susceptible to infections, but we found no correlation between IgE elevation and bacterial infections (Ringdén, unpublished observations).

We can only speculate about the mechanisms underlying the increase in IgE concentrations. Antigenic stimulation may lead to an increase in the total IgE level. In our patients, minor transplantation antigens or cell surface components modulated by virus may have served as stimulating determinants. Foreign histocompatibility antigens present on leukocytes contained in transfused blood are other possible sources of stimulating determinants. The finding of IgE antibodies in the patient who received marrow from her monozygous twin may be explained by the latter possibilities. The magnitude of the IgE response was extremely high in some patients, indicating a polyclonal activation induced by an unknown stimulus.

Cells with helper and suppressive functions play a vital role in the regulation of antigen-induced IgE responses. Therefore, the increase in IgE levels in bone-marrow-transplanted patients may be due to disturbance of the regulatory functions. Prior to transplantation, the patients are treated with cytotoxic drugs, with or without total body irradiation. This treatment has been shown to lead to an enhanced antigen-induced IgE response in mice and rats, which is probably due to the loss of radiosensitive suppressive T cells. Another possibility is a delayed maturation of regulatory T cells from recipients in the bone marrow graft. In most patients, regardless of the immunosuppressive treatment of acute GVHD, the increase in IgE was followed by a rapid decrease, indicating a rapid turn-off of activated B cells. The half-life of serum IgE has been estimated earlier at 2.7 days.

Reinherz et al. have reported that bone-marrow-transplanted patients subjected to acute GVHD lack TH₁ cells in peripheral blood, a subpopulation of T lymphocytes containing cells with cytotoxic and suppressive functions. However, in other extended studies using monoclonal antibodies for the characterization of T subsets, the proportion of T8⁺ or Leu-2⁺ cells (containing suppressor/cytotoxic T cells) were normal or high and the proportion of helper/inducer cells was low early after transplantation, regardless of acute GVHD. Obviously, the increase in IgE levels occurred during a period with decreased helper/inducer T cells in the blood. These findings are not contradictory, since T-cell subsets in the blood may not be representative of T cells in all lymphoid organs. Furthermore, it is unknown at present if these surface markers define T cells involved in the regulation of IgE.

It seems that most bone marrow transplant recipients who are observed frequently and long enough have a period of elevated IgE, regardless of clinical GVHD. In any case, in patients with GVHD, the timing of this elevation and acute GVHD is significant.

**ACKNOWLEDGMENT**

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