Defective Interleukin 2 Production in Patients After Bone Marrow Transplantation and In Vitro Restoration of Defective T Lymphocyte Proliferation by Highly Purified Interleukin 2

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Using OKT3 monoclonal antibody as a mitogen, we have studied interleukin 2 (IL2) production and proliferation in peripheral blood mononuclear cells (PBMC) of 23 patients receiving bone marrow transplants. Twenty patients were recipients of allogeneic bone marrow for treatment of hematologic malignancies, aplastic anemias (AA), or severe combined immunodeficiencies (SCID). Three patients with Hodgkin’s disease or neuroblastoma received autologous bone marrow. Endogenous IL2 production was not detectable (< 0.2 U/mL) in PBMC of 18 patients and was very low in PBMC from five patients (0.5 to 1.5 U/mL), as compared to normal controls (median 3.5 U/mL) or pretransplant patients (median 1.5 U/mL). The low IL2 production was associated with defective OKT3-induced proliferation of PBMC in 19 of 23 patients studied. In the first 6 months after BMT, 14 of 15 patients (93%) showed defective proliferation of PBMC as compared to five of eight patients (63%) tested between 7 and 18 months after BMT (P < .1). In all but three patients, addition of highly purified human lymphocyte IL2 (hpIL2) restored OKT3-induced proliferation of PBMC to within the normal range. This study demonstrates that PBMC in patients after BMT have a defect of IL2 production but are able to express IL2 receptors in response to OKT3 antibody and to proliferate normally upon addition of hpIL2. PBMC of all patients showed similar functional defects, whether or not they received additional therapy, including various conditioning regimens prior to BMT and immunosuppressive therapy after BMT. These observations suggest that T cell defects after BMT are most likely secondary to quantitative or qualitative defects of transplanted T lymphocytes or their precursors.

During the first two years after transplantation, recipients of allogeneic marrow transplants demonstrate a severe deficiency of cellular and humoral immunity. This immunodeficiency leads to increased susceptibility to frequently lethal bacterial, fungal, and viral infections.

Interleukin 2 (IL2) is one of the major cytokines responsible for clonal expansion of T cells and human natural killer (NK) cells, as well as for the activation of cytotoxic effector cells. The role of IL2 in several immunodeficiency states as well as in certain lymphoid leukemias has been previously documented. Defective IL2 production could be one possible mechanism for the abnormal immune function in patients after bone marrow transplantation (BMT).

Recently, OKT3, a monoclonal antibody against a T cell surface antigen, has been shown to be mitogenic, even in nanogram concentrations, and to induce IL2 production in total T cells as well as in T cell subsets. OKT3 appears to recognize an epitope of the antigen-recognition complex on T lymphocytes, triggering mitogenesis in a way similar to that induced by antigen.

We studied IL2 production and response of peripheral blood mononuclear cells (PBMC) from patients up to 18 months after BMT using OKT3 antibody as mitogen and, as control, phytohemagglutinin (PHA). In addition, we investigated whether highly purified human lymphocyte IL2 (hpIL2) was capable of restoring defective proliferative responses of T cells in vitro.

Materials and Methods

Patients

The study group consisted of 20 recipients of an allogeneic BMT: four patients with acute myelogenous leukemia (AML) in first or second remission, four patients with acute lymphoblastic leukemia (ALL) in second or third remission, four patients with chronic myelogenous leukemia (CML), six patients with aplastic anemia (AA), and two patients with severe combined immunodeficiency (SCID). In addition, three recipients of autologous BMT were studied: one patient with neuroblastoma and two patients with non-Hodgkin’s lymphoma (NHL). Details of the transplantation procedure have been described. Patients with AML, ALL, and CML were conditioned for transplantation with cyclophosphamide (60 mg/kg for two days) and hyperfractionated total body irradiation (TBI, total dose 1,320 rad). In the aplastic anemia group, four patients received only preparative chemotherapy with cyclophosphamide (50 mg/kg for four days), cytosine arabinoside (200 mg/kg/d for five days) and 6-thioguanine (200 mg/kg/d for five days), whereas two other patients were conditioned similar to patients with leukemias but with less TBI (800 and 300 rad, respectively). SCID patients received no cytoreductive treatment. The patient with neuroblastoma was treated with L-phenylalanine mustard (L-PAM) 240 mg/m² plus dihydrOGalactitol 240 mg/m² and low TBI, while the two patients with NHL received the same regimen used for the
ID2 PRODUCTION AND RESPONSE AFTER BMT

patients with leukemias. All patients received an allogeneic BMT, except one, received the marrow from HLA-A, B, C, and D identical siblings and engrafted permanently following the first attempt. One patient with SCID engrafted permanently only after the fourth attempt, when receiving a lectin-separated marrow from his haploidentical mother. Posttransplant immune suppression for all patients receiving allogeneic BMT consisted of methotrexate, 15 mg/m2 on day 1, followed by 10 mg/m2 on days 3, 6, 13, 20, and weekly thereafter to day 100. All immune suppressive drugs were stopped at that time. Patients with graft-versus-host disease (GVHD) of at least grade 2 were treated with high-dose prednisone (2 mg/kg/d). At the time of the IL2 analysis, 13 patients had GVHD (four patients grade 1; three patients grade 2; and six patients grade 3). Four of the 13 patients had acute GVHD.

Proliferation Assay

Mononuclear cells (PBMC) from 10 to 20 mL of heparinized blood were separated by density gradient centrifugation on Ficoll-Hypaque as previously described and resuspended at a final concentration of 106 cells/mL in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), glutamine (2 mmol/L), penicillin (50 U/mL), and streptomycin (50 μg/mL). Each sample was stimulated in triplicate microwell cultures (No. 3596 culture plate, Costar Inc., Cambridge, Mass) in the presence or absence of mitogen (PHA, OKT3), and in the presence or absence of hpIL2. Unless stated otherwise, the concentrations used were 1.25 ng/mL for OKT3 (Ortho Pharmaceuticals, Raritan, NJ) and 0.5% (vol/vol) for PHA (PHA-M, Grand Island Biological Co., Grand Island, NY). After 24 hours, 100 μL of supernatant was removed from each well to be assayed for IL2 activity. Identical cultures were incubated for three days and then pulsed for four hours with 1H-thymidine (0.5 μCi/well; New England Nuclear, Boston), and the 3H-thymidine uptake measured as described previously. Maximum IL2 production was seen at 24 hours, and maximum proliferation was seen after three days. Interleukin 1 (IL1) was purchased from Genzyme, Co. (Norwalk, Conn), and its effect on IL2 production and lymphocyte proliferation was tested in PBMC cultures from two patients. Statistical evaluation was performed using the Kruskal-Wallis test (two-sided P values).

Assays for IL2 Activity

For the IL2 assay, IL2-dependent cultures of cytotoxic T lymphocytes were used as previously described. Preparion of Purified Human IL2

The protocol for purification of human IL2 to apparent homogeneity has been described in detail. Briefly, PBMC were suspended in RPMI 1640 (GIBCO) with 0.25% human serum albumin and 1% PHA (GIBCO) at a concentration of 4 x 106 cells/mL for 48 hours at 37 °C and costimulated with Daudi cells. IL2 from this conditioned medium was purified by using sequential ammonium sulfate precipitation, ion-exchange chromatography (DEAE cellulose, DE 52, Whatman, Clifton, NJ), gel-filtration (A24 54 Ultrogel, LKB Instruments, Rockland, Md), chromatography on blue agarose (BRL, Gaithersburg, Md), and chromatography on Procion-red agarose (BRL). The final product had a specific activity of 106 U/mg protein and lacked detectable interferon (alpha and gamma), granulocyte-macrophage colony-stimulating factor, B cell growth factor, B cell differentiating factor, and thymocyte differentiating factor activities.

RESULTS

IL2 Production

Table 1 details the IL2 production data of the patient population. PBMC from 18 patients after BMT did not produce detectable amounts of IL2 (less than 0.2 U/mL). PBMC from five patients produced between 0.5 and 1.5 U/mL IL2, as compared to a median of 3.5 U/mL IL2 in controls (n = 21) (P < .001), when stimulated with OKT3 antibody. IL2 production was not significantly different between the different diagnostic subgroups (P > .1). PBMC from eight patients (all diagnostic subgroups) analyzed prior to BMT produced IL2 in the low normal range (Table 1).

Proliferative Response to OKT3 Antibody and PHA

As shown in Figs 1 through 3, low IL2 production was followed by a low proliferative response of PBMC, as measured by 3H-thymidine incorporation on day 3. PBMC from nine of the 12 patients with leukemias, five of six patients with AA, both SCID patients, and the three patients after autologous BMT had defective mitogen responses to OKT3 (Fig 1) as compared to normal controls (n = 21). The PBMC from eight

Table 1. OKT3- and PHA-Induced IL2 Production of PBMC From Patients After BMT

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>OKT3 (1.25 ng/mL) IL2 (U/mL) Median (Range)</th>
<th>PHA (0.5%, vol/vol) IL2 (U/mL) Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td>12</td>
<td>&lt; 0.2 (&lt; 0.2 - 1.5)</td>
<td>&lt; 0.2 (&lt; 0.2 - 1.0)</td>
</tr>
<tr>
<td>(ALL, AML, CML)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>6</td>
<td>0.25 (&lt; 0.2 - 1.0)</td>
<td>&lt; 0.2 (all &lt; 0.2)</td>
</tr>
<tr>
<td>Severe combined immunodeficiency</td>
<td>2</td>
<td>&lt; 0.2 (both &lt; 0.2)</td>
<td>&lt; 0.2 (both &lt; 0.2)</td>
</tr>
<tr>
<td>Autologous BMT (NHL, neuroblastoma)</td>
<td>3</td>
<td>&lt; 0.2 (all &lt; 0.2)</td>
<td>&lt; 0.2 (all &lt; 0.2)</td>
</tr>
<tr>
<td>Controls</td>
<td>21</td>
<td>3.5 (0.9 - 16)</td>
<td>2.3 (0.8 - 6.8)</td>
</tr>
<tr>
<td>(A) Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B) Prior BMT*</td>
<td>8</td>
<td>1.5 (0.4 - 2.5)</td>
<td>1.0 (0.7 - 1.6)</td>
</tr>
</tbody>
</table>

*Patients prior to BMT (all diagnostic subgroups).
Fig 1. OKT3-induced proliferation of PBL from patients of different diagnostic subgroups after BMT in the presence or absence of highly purified IL2. Cell proliferation was measured by 3H-thymidine incorporation on day 3 of culture in the presence (○) or absence (●) of highly purified IL2. The shaded area represents the 100% range of normal donors (n = 21) in the presence or absence of highly purified IL2. (●●) Patients with GVHD; (—) median 3H-thymidine uptake.

patients (all diagnostic subgroups) tested prior to BMT showed a wide range of proliferative responses to OKT3 antibody (median, 38,000 cpm; range, 15,000 to 88,000 cpm), and to PHA (median, 71,000; range, 17,000 to 165,000 cpm), respectively. These responses were significantly higher than those seen after BMT (P < .05). Whereas the median proliferative response of PBMC from patients after BMT in the absence of IL2 was similar for OKT3 and PHA as mitogens, the median 3H-thymidine incorporation in the presence of hpIL2 was much higher when OKT3 (median 50,500 cpm) rather than PHA was used as mitogen (median 24,500 cpm) (Fig 2). In all except three patients, proliferation of PBMC could be restored to within the normal range by hpIL2 (Figs 1 through 3) when OKT3 was used as mitogen. The enhancement of proliferation by addition of hpIL2 (10 U/mL) to OKT3- or PHA-stimulated PBMC was significantly higher in patients (OKT3: median 6.8-fold; PHA: 3.4-fold) than in normal controls (OKT3: median 1.34-fold; PHA: 1.13-fold) (P < .001). However, this enhancement was higher for OKT3 than for PHA (P < .005; comparing IL2 enhancement ratios in patients vs normal controls). To test whether defective production of IL1 by monocytes was responsible for the low endogenous IL2 production and proliferative responses of PBMC from the BMT patients, IL1 (up to 100 U/mL) was added to the mitogen-stimulated cultures of two patients. No effect was seen, both in the absence and presence of exogenous IL2.

Studies in controls and six patients after BMT with OKT4/OKT8 T cell ratios of less than 1.0 showed that the ratio of T lymphocyte subsets did not change in vitro when cultured for three days in the presence of OKT3 antibody and hpIL2 (data not shown). This finding suggests that there was no preferential growth of one subpopulation of T cells under our experimental conditions.

The Effect of Time From BMT on Mitogen Responses to OKT3 Antibody

Figure 3 demonstrates that a proliferative defect was present even up to 18 months after BMT. However, the median proliferation of PBMC in absence of hpIL2 was higher (12,000 cpm) in the group 7 to 18 months after BMT than in the group in the first six months after BMT (5,800 cpm) (P = .09). In the presence of hpIL2, the median 3H-thymidine uptake of PBMC was 79,000 cpm in the seven- to 18-month group and 41,000 cpm in the group less than seven months after BMT (P = .06).

The Effect of Conditioning Regimens on Mitogen Responses to OKT3 Antibody

IL2 production and proliferation of PBMC from patients receiving no conditioning regimen (SCID),
chemotherapy alone (four patients with AA) or receiving chemotherapy and TBI (1,320 rad for patients with leukemia or receiving autologous transplant; 300 or 800 rad for two patients with AA) were compared. No differences between these groups were seen with respect to endogenous IL2 production or proliferative responses to OKT3 or PHA, respectively, in the absence or presence of exogenous hpIL2 (Fig 1).

The Effect of GVHD and Immunosuppressive Drugs on Mitogen Responses to OKT3 Antibody

The study population consisted of three groups with respect to immunosuppressive medications given after BMT. Patients receiving autologous BMT were given no further immunosuppressive therapy after BMT. All patients receiving allogeneic BMT were treated with prophyllactic methotrexate, while those with GVHD received, in addition, high-dose prednisone (see Materials and Methods). Only one patient received prednisone plus cyclosporine A for GVHD. No differences between groups were seen with respect to endogenous IL2 production (Table 1) and proliferative responses to OKT3 antibody and PHA, respectively, in the absence or presence of exogenous IL2 (Fig 1). It is interesting that PBMC from the one patient receiving cyclosporine A showed the lowest mitogen responses of all patients tested (OKT3 response without IL2: 1,100 cpm; with exogenous hpIL2: 13,000 cpm).

The study group included 13 patients who developed acute or chronic GVHD (grade 1 to 3) (shown with asterisks beside the symbols in Fig 1). There were no statistically significant differences in the mitogen responses nor in the restoration of proliferation of PBMC by hpIL2 between patients with or without GVHD.

DISCUSSION

This study demonstrates that PBMC from patients up to 18 months after BMT have defective mitogen responses that are secondary to a defect in IL2 production. This finding is specific for patients after BMT, as PBMC from patients tested prior to BMT do not exhibit this defect to the same degree. Addition of IL1 to mitogen-stimulated PBMC cultures of two patients was without effect on IL2 production and proliferation, suggesting that defective IL1 production by monocytes is not responsible for the observed defects. Most of the patients had an imbalance in the OKT4/OKT8 (Leu-3/Leu-2) T cell ratio, with a predominant OKT8+ subpopulation (unpublished observations). However, using OKT3 as mitogen, both subgroups (OKT4+, OKT8+) of T lymphocytes were capable of producing IL2. It is therefore unlikely that the reversed OKT4/OKT8 ratio is the reason for the IL2 production defect. In addition, we have not found any correlation between IL2 production by PBMC and OKT4/OKT8 T cell ratio in ten patients tested. The lack of correlation between low OKT4/OKT8 T cell ratios and OKT3 mitogen responses has recently been documented in other immunodeficiency states, including patients with acquired immunodeficiency syndrome (AIDS). Other factors that could have been reasons for defective IL2 production by PBMC include: (A) the quality or quantity of transplanted T lymphocytes and their precursors, (B) the conditioning for the transplantation with alteration of the microenvironment by chemotherapy and irradiation, (C) posttransplant immunosuppression (prednisone, methotrexate), (D) T cell maturation defects following the absence of a normal thymic milieu (factors A through D have also been proposed as possibly causing the low OKT4/OKT8 ratio), and (E) a defective interaction between Ia+ monocytes/macrophages and T cells. Because similar defective OKT3 mitogen responses were seen following autologous and allogeneic BMT, it appears unlikely that histocompatibility differences or posttransplant immunosuppressive therapy were responsible for the defects. Furthermore, patients receiving (A) no conditioning regimen or (B) chemotherapy with or without TBI showed the same defects with respect to OKT3 mitogen responses. Although several of the agents given to BMT patients have previously been shown to suppress IL2 production and response by PBL (eg, cyclophosphamide, cyclosporine A), no clear relationship between administration of these drugs and defective OKT3 mitogen responses was observed. These observations would suggest that defects in T cell function following BMT were primarily due to the quality or quantity of transplanted T lymphocytes or their precursors. This hypothesis is in agreement with previous reports demonstrating relative immaturity of T lymphocytes in BMT patients.

As expected, the defective IL2 production was followed by defective proliferation of PBMC as measured by [3H]-thymidine incorporation. In the first six months after BMT, PBMC from all patients but one exhibited defective mitogen responses to OKT3. In the group 7 to 18 months after BMT, PBMC from three of eight patients showed normal proliferative responses. In the presence of hpIL2 (10 U/mL), proliferative responses of PBMC to OKT3 antibody could be restored to within the normal range in all but three patients. One of these three patients received cyclosporine A at the time of study. However, his PBMC proliferation improved from 1,100 cpm in the absence of IL2 to 13,000 cpm in the presence of IL2. PBMC from the two other patients showed normal proliferation upon addition of hpIL2 two months after the first study.
Whether different kinetics of restoration of IL2 production and T cell proliferation after BMT are of prognostic value is under investigation.

The finding that OKT3 antibody was a potent mitogen raised the possibility that OKT3 antibody was reacting with the antigen recognition complex of T cells, triggering mitogenesis in a way similar to that induced by antigen. The percentage of OKT3-positive lymphocytes reaches normal values shortly after transplantation in most patients and remains normal thereafter in all patients. Possible induction of OKT3 cells, triggering mitogenesis in a way similar to that induced by antigen, may be part of the mitogenic effect of OKT3 antibody, within a few hours (E. Rinnooy Kan, E. Platzer, K. Welte, C.Y. Wang, submitted for publication). The OKT3 antigen modulation, however, does not render cells unresponsive to IL2.

In this article, we have documented that the OKT3 antibody-induced proliferation of PBMC was normalized in vitro in nearly all patients by hpIL2, whereas only four patients achieved normalization of their proliferative response of PBL upon addition of hpIL2 with PHA as mitogen. This observation would suggest that T lymphocytes in patients after BMT were able to express normal levels of IL2 receptors in response to OKT3 antibody but not to PHA. IL2 has previously been shown to be able to restore (A) impaired cell-mediated lympholysis in patients with acute GVHD but not chronic GVHD and (B) PHA-stimulated T cell colony formation of lymphocytes from patients early after BMT. Both groups hypothesized that the defect in those patients was a functional T helper cell defect. Both reports are consistent with our observations that T cells from patients after BMT were capable of responding to IL2.

As defective IL2 production and correction of functional T cell defects in vitro by hpIL2 have now been well-documented in patients after BMT, in vivo administration of IL2 might be beneficial for these patients. In our ongoing phase I trial of hpIL2 in patients with other immunodeficiency states (AIDS, lymphoma), no side effects have been seen at up to 20,000 U/m2 for 14 days. However, in patients after BMT, IL2 might enhance or cause acute GVHD. Animal studies have been initiated to address this problem.

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REFERENCES


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