Serum Interleukin-3 Levels Following Autologous or Allogeneic Bone Marrow Transplantation: Effects of T-Cell Depletion, Blood Stem Cell Infusion, and Hematopoietic Growth Factor Treatment

By Kenneth F. Mangan, Mark T. Mullaney, Teresa Díaz Barrientos, and Nancy A. Kernan

Engraftment of marrow following autologous or allogeneic bone marrow transplantation (BMT) may be influenced by quantity and function of stem cells, T lymphocytes, supporting microenvironmental cells, and hematopoietic growth factors (HGF). To elucidate the physiologic role of interleukin-3 (IL-3) in the engraftment process, serum IL-3 levels were measured in over 400 samples from 77 transplant recipients before and for up to 3 weeks following transplantation using a novel enzyme-linked immunosorbent assay (ELISA) with a sensitivity of ≥78 pg/mL. Thirty-seven patients received two to three log T-cell-depleted allografts. In the remaining 40 patients (18 autologous marrow, 12 allogeneic marrow, and 10 autologous peripheral blood [PB] stem cell), T cells were not depleted (non-TCD) from the grafts. A burst of IL-3 (peak levels, 1,500 to 8,000 pg/mL) was detected in the immediate posttransplant period between day 0 and day 14 in all non-TCD recipients and in 21 of 37 (57%) of TCD recipients. A strong inverse relationship between IL-3 levels and absolute neutrophil count (ANC) was observed in both non-TCD recipients (r = -.796) and in TCD recipients (r = -.897).

However, both peak IL-3 levels and mean IL-3 levels from day 0 through day 14 were significantly lower in TCD recipients compared with either autologous or unmodified allogeneic marrow recipients (P < .01). The lowest peak or mean day 0 through day 14 IL-3 levels were observed in matched related recipients undergoing the most aggressive (2.5 to 3.0 log) T-cell–depleted BMT. Autografted patients receiving blood stem cell transplants alone or posttransplant granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF) also had significantly lower peak IL-3 levels (P < .01). In patients receiving TCD grafts, administration of antithymocyte globulin (ATG) posttransplant significantly increased peak IL-3 levels compared with patients not treated with ATG (P < .04). This study shows that endogenous release of IL-3 is strongly associated with myeloid engraftment and inversely related to ANC. Removal of T lymphocytes from donor marrow or acceleration of engraftment by use of stem cells or growth factors appears to blunt the endogenous release of IL-3 whereas use of ATG posttransplant increases IL-3 release.

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Materials and Methods

Informed consent for blood samples was obtained from the patients and normal volunteers as approved by the Institutional Review Boards of Temple University Health Sciences Center and Memorial Sloan Kettering Cancer Center.

Transplant patients. The clinical characteristics of the 77 transplant recipients included in this study are summarized in Tables 1 and 2. Eighteen autologous BM, 10 autologous peripheral blood stem cell (PBSC), 12 unmodified allogeneic marrow (Table 1), and 37 modified or TCD allografts. The results show that a predictable IL-3 burst is observed in the early posttransplant period when absolute neutrophil count drops below 1,000/μL.

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A. Autologous BMT

C. Allogeneic BMT

cyclophosphamide (6 g/m²), carboplatin (1,200 mg/m²), thiotepa (500 mg/m²). or etoposide (2.4 g/m²); CEP, cyclophosphamide (6 g/m²), etoposide (100 pg/mL) or VP-16 (30 pg).

Busulfan (1 6 mg/kg) and cyclosphosphamide (200 mg/kg); Cy/TBI, cyclophosphamide (120 mg/kg) and total body irradiation (200 cGy x 6 = 1,200 cGy); Cy only, cyclophosphamide (200 mg/kg); CPT or CPE, cyclophosphamide (6 g/m²). or etoposide (2.4 g/m²); CEP, cyclophosphamide (6 g/m²), etoposide (2.4 g/m²), cisplatin (150 mg/m²).

Five patients also received autologous GM-CSF mobilized pbsc with autologous bone marrow.

Five patients had BM purged with 4-hydroperoxycyclophosphamide (100 μg/mL) or VP-16 (30 μm). GVHD prophylaxis consisted of continuous infusion cyclosporin (3 mg/kg/d) and four doses of methotrexate at days +1, +3, +6, and +11.

Table 1. Characteristics of Recipients of Autologous Bone Marrow or Peripheral Blood Stem Cell Transplants and Unmodified Allogeneic Bone Marrow Transplants

<table>
<thead>
<tr>
<th>Types of BMT and Diagnosis</th>
<th>N</th>
<th>Cytoreduction</th>
<th>Age (yrs) Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Autologous BMT†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 3-4 breast</td>
<td>6</td>
<td>Bu/Cy</td>
<td>44 (22–80)</td>
</tr>
<tr>
<td>Stage 3-4 breast</td>
<td>4</td>
<td>CPT or CPE</td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>3</td>
<td>Cy/TBI</td>
<td></td>
</tr>
<tr>
<td>Stage 3-4 NHL</td>
<td>2</td>
<td>CEP</td>
<td></td>
</tr>
<tr>
<td>AML rem</td>
<td>2</td>
<td>Bu/Cy</td>
<td></td>
</tr>
<tr>
<td>Stage 4 NHL</td>
<td>1</td>
<td>TBI/VP</td>
<td></td>
</tr>
<tr>
<td>B. Autologous PBSC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 4 NHL</td>
<td>5</td>
<td>CEP or CEC</td>
<td>41 (26–56)</td>
</tr>
<tr>
<td>Stage 4 breast</td>
<td>2</td>
<td>Bu/CY</td>
<td></td>
</tr>
<tr>
<td>Stage 4 breast</td>
<td>1</td>
<td>CEC</td>
<td></td>
</tr>
<tr>
<td>Stage 4 HD</td>
<td>1</td>
<td>CEP</td>
<td></td>
</tr>
<tr>
<td>C. Allogeneic BMT†</td>
<td></td>
<td></td>
<td>29 (18–49)</td>
</tr>
<tr>
<td>AML rem</td>
<td>6</td>
<td>Bu/Cy</td>
<td></td>
</tr>
<tr>
<td>CML-CF/AP</td>
<td>3</td>
<td>Bu/Cy</td>
<td></td>
</tr>
<tr>
<td>SAA</td>
<td>2</td>
<td>Cy only</td>
<td></td>
</tr>
<tr>
<td>AML-rem</td>
<td>1</td>
<td>Cy/TBI</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BMT, bone marrow transplant; PBSC, peripheral blood stem cell transplant; Ca, cancer; MM, multiple myeloma; NHL, non-Hodgkin’s lymphoma; AML-rem, acute myelogenous leukemia in remission; HD, Hodgkin’s disease; CML-CP/AP, chronic myelogenous leukemia in chronic or accelerated phase; SAA, severe aplastic anemia; Bu/Cy, busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg); Cy/TBI, cyclophosphamide (120 mg/kg) and total body irradiation (200 cGy x 6 = 1,200 cGy); Cy only, cyclophosphamide (200 mg/kg); CPT or CPE, cyclophosphamide (6 g/m²). or etoposide (2.4 g/m²); CEP, cyclophosphamide (6 g/m²), etoposide (2.4 g/m²), cisplatin (150 mg/m²).

Table 2. Characteristics of Recipients of TCD BM Transplants

<table>
<thead>
<tr>
<th>Type of BMT and Diagnosis</th>
<th>N</th>
<th>Cytoreduction</th>
<th>Age (yrs) Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. HLA-Identical Family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Member Donor†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBA-E</td>
<td>15</td>
<td>25 (6–77)</td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>4</td>
<td>TBI + CTX + VP16</td>
<td></td>
</tr>
<tr>
<td>AML-rem</td>
<td>2</td>
<td>TBI + CTX + AZQ</td>
<td></td>
</tr>
<tr>
<td>ALL-rem</td>
<td>3</td>
<td>TBI + VP16 + TH</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>2</td>
<td>TBI + CTX</td>
<td></td>
</tr>
<tr>
<td>SBA-CDS/8†</td>
<td>12</td>
<td>33 (22–53)</td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>10</td>
<td>TBI + CTX + TH or VP16</td>
<td></td>
</tr>
<tr>
<td>ALL-rem</td>
<td>1</td>
<td>TBI + CTX + TH</td>
<td></td>
</tr>
<tr>
<td>AML-rem</td>
<td>1</td>
<td>TBI + CTX + TH</td>
<td></td>
</tr>
<tr>
<td>B. Closely HLA-matched</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unrelated Donor*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBA-E</td>
<td>10</td>
<td>22 (7–37)</td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>5</td>
<td>TBI + CTX + TH or VP16</td>
<td></td>
</tr>
<tr>
<td>ALL-rem</td>
<td>4</td>
<td>TBI + CTX + TH or VP16</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1</td>
<td>TBI + CTX</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SBA E, soybean agglutinin and E-rosette depletion; SBA-CDS/8, soybean agglutinin and MoAb depletion (anti-CDS and anti-C8); CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; AA, aplastic anemia; rem, remission; rel, relapse; TBI, total body irradiation (1,500 cGy); VP16, 250 mg/m² x 3 doses; CTX, cyclooxan 80 mg/kg x 2 doses; TH, thiotepa, 200 mg/m² x 3 doses (ALL) or 5 mg/kg x 2 doses (CML); AZQ, 8 mg/m² x 7 doses.

Seventeen of the 27 recipients of HLA-identical marrow received antithymocyte globulin 15 mg/kg every other day from day +5 to +19 posttransplant and methylprednisolone 2 mg/kg to promote engraftment. All 10 recipients of related marrow received this rejection prophylaxis regimen.

TCD allogeneic marrow transplant recipients (Table 2) received various myeloablative conditioning regimens for hematologic malignancies, aplastic anemia, or advanced breast cancer. Fifteen of 28 autologous marrow or blood stem cell recipients also received posttransplant granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage CSF (GM-CSF) to accelerate engraftment. Graft-versus-host disease prophylaxis included cyclosporine (3 mg/kg continuous infusion) and four doses of intravenous methotrexate at days +1, +3, +6, and +11.

were administered prophylactic antibiotics and Igs and were treated in laminar air flow or high-efficiency particulate-arresting (HEPA) air-filtered rooms. Patients were maintained by transfusions to greater than 24% hematocrit and to greater than 20,000/mm³ platelets. The mean time to absolute neutrophil count (ANC) 500/µL was 14 days for PBSC transplants and 19 days for either autologous or unmodified allogeneic marrow transplant recipients. Patients receiving TCD transplants engrafted after a mean of 16 days.

Serum samples. To establish optimal time for detecting IL-3, daily or every other day serum samples were retrieved before cytoreductive therapy and for up to 3 weeks following marrow or stem cell infusions in at least two patients representing each of the following five transplant types: autologous marrow, autologous PBSC, unmodified allogeneic marrow, TCD allogeneic marrow by SBA-E method, and TCD allogeneic marrow by SBA-CDS/8 method. Because serum IL-3 peak release was always observed between days 0 and +14 when ANC was less than 1,000/µL, at least two serum samples were tested in all subsequent transplant recipients between days 0 and +14. A total of 254 serum samples were tested in the 77 recipients between days 0 and day +14. Another 168 samples were tested either before day 0 or after day +14. All samples were stored frozen at -80°C and thawed once before assay in the ELISA.
IL-3 ELISA. Individual wells of a 96-well microtiter dish (Dynatech Immulon 1 plates; Fisher-Scientific, Pittsburgh, PA) were coated with 0.1 mL of 0.5 μg monoclonal murine anti-human IL-3, “first antibody,” (Genzyme, Boston, MA) which had been diluted with phosphate-buffered saline (PBS), pH 7.3. The plate was covered, centrifuged for 2 minutes at 2,000 rpm, and incubated overnight at 4°C. The plate was washed three times with a 0.2 mL volume of washing solution (0.01% Tween 20 [Sigma Chemical Co, St Louis, MO]) in PBS using a multichannel pipetter (GIBCO, Grand Island, NY) inverted, and dried with tissue paper. All washings described below followed this same procedure. Recombinant human IL-3 (Amgen, Thousand Oaks, CA, or Genzyme) used as the standard was diluted in blocking buffer (0.25% bovine serum albumin (BSA) in PBS). To duplicate wells 0.1 mL recombinant human IL-3 (rhIL-3) corresponding to 0, 0.5, 1, 2, 5, 10, 50, 150, 500 pg/mL IL-3 were retested at different dilutions. The plate was incubated for 2 hours at 37°C. After aspiration and washing as described above, 1.0 μg in 0.1 mL blocking buffer of polyclonal rabbit anti-human IL-3 “second antibody,” (Genzyme) was added to all the wells. The plate was incubated at 37°C for 2 hours. After aspiration and three washings, 2.0 ng in 0.1 mL biotin-conjugated goat anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA) diluted 1:1,000 in blocking buffer was added to each well. The plate was incubated at 37°C for 45 minutes followed with three washes. Next, 0.1 mL of 1.0 μg/mL peroxidase-conjugated streptavidin (Jackson Immunoresearch) was added with incubation at 37°C for 40 minutes. The plate was then washed five times. Within 30 minutes of use (during the last incubation) the reaction solution consisting of 0.1 mol/L phosphate citrate buffer, pH 5.0, 0.4 mg/mL O-Phenylenediamine dihydrochloride (OPD) (Sigma), and 0.015% H2O2 was made. A 0.1 mL volume of this solution was then added to each well and the reaction was allowed to proceed for 5 minutes at room temperature. The color development was stopped with 0.1 mL of 1N H2SO4. The plate was read on a microplate reader (2550 ELA Reader, Biorad, Rockville Centre, NY) with the absorbance set at 492 nm. The concentration of IL-3 in a test sample was then obtained from a curve plotted from the standards.

MTT bioassay for IL-3. The human megakaryoblastic leukemia cell line M-07E (kindly provided by Dr James Ihle, St Jude Children’s Hospital, Memphis, TN, with permission from Dr PegoRaro, Torino, Italy) detects IL-3 and GM-CSF in human serum and cell culture media.11 In preliminary experiments we confirmed that recombinant IL-3 increased proliferation and δ-thymidine uptake in M07E cells and this uptake was abrogated by addition of anti-IL-3 monoclonal antibody. The cells were maintained at 4 x 10^5 cells/mL in 5% CO2 and re-fed every 3 to 4 days with Dulbecco’s Minimal Essential Medium (DMEM) (GIBCO, Grand Island, NY) supplemented with 4.5 g/L glucose, 200 mmol/L L-glutamine, 100 mmol/L sodium pyruvate (sDMEM), 20% horse serum (HS), and 10% U/mL recombinant human IL-3 (rhIL-3). Before assay, the cells were washed three times in sDMEM, 10% HS. In a 96-well microtiter dish, 0.1 mL of the cell suspension (1 x 10^5 cells) and 0.1 mL of sample (neat, diluted) were added to all wells. Test serum diluted 1:10 were preincubated with anti-GM-CSF monoclonal antibodies (MoAbs) (R & D Systems, Minneapolis, MN) to block GM-CSF effects. Duplicate samples were incubated at 37°C, 5% CO2. On day 3 of culture 0.1 mg (20 μL of 5 mg/mL solution) of freshly made MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma Chemical Co, St Louis, MO) was added to each well.12 The plate was incubated for 4 hours at 37°C, 5% CO2. The culture plate was then centrifuged at 1,000 rpm for 5 minutes to pellet the cells. The media was carefully aspirated

and 100 μL dimethyl sulfoxide (DMSO) (Research Industries, Salt Lake City, UT) was added to each well followed by gentle agitation to dissolve the formazan crystals. The plate was then read on the microplate reader with the absorbance set at 562 nm.

Source of growth factors. The 18 human recombinant growth factors cross-checked in the ELISA were obtained from Amgen (IL-2, G-CSF, GM-CSF, erythropoietin and interferon [IFN]-γ), Cistron, Pine Brook, NJ (IL-1 β), Genetics Institute, Cambridge, MA (IL-9), Genzyme, Cambridge, MA (IL-10, M-CSF, c-kit ligand), Hoffman-LaRoche, Nutley, NJ (IFN-α), and R & D Systems, Minneapolis MN (IL-4, IL-5, IL-6, IL-7, IL-8, transforming growth factor δ [TGF δ], tumor necrosis factor α [TNF α]).

Statistics. Analysis between cohorts were made using the Student’s two-tailed t-test.

RESULTS

Effects of sera in the ELISA. The ELISA used three different commercially available antibodies (monoclonal murine anti-human IL-3, polyclonal rabbit anti-human IL-3, and goat-anti-rabbit IgG) in a typical sandwich approach. Standard curves (Fig 1) for the ELISA for human IL-3 were constructed in the presence (solid dots) and absence of 10% type AB serum (open squares). As shown in Fig 1, 10% serum had no significant effects on absorbance values in comparison with curves generated without sera (P > .52). The curves were linear below 1,250 pg/mL (R2 = 0.956) and could reliably detect as little as 78 pg/mL IL-3 in the sample. Since high concentrations of human sera can nonspecifically interfere in ELISAs,13,14 we tested various concentrations of sera from three separate donors. Recovery of exogenous IL-3 was greater than 95% (in comparison with PBS only controls) in the presence of 10% sera. However, IL-3 recovery decreased to 55% in the presence of 25% sera and it was undetectable when serum concentrations increased to greater than 50%. Thus, a dilution of human serum 10-fold was required to reliably detect IL-3 in the ELISA. Under these conditions, the assay was reproducible and sensitive to a level of 78 pg/mL in test samples with an interassay variation of 4.5% to 15.6%.

Validation of the ELISA. In the absence of the second antibody (polyclonal rabbit anti-human IL-3) or third antibody (biotin-conjugated goat-anti-rabbit IgG), the absorbance of the wells was equal to that of unconjugated blanking wells, indicating extremely low nonspecific binding of both antibodies. In addition, when serially diluted rabbit anti-IL-3 antibody was mixed with various dilutions of recombinant IL-3, color development in the ELISA was inhibited in a dose-dependent fashion (data not shown) confirming its dependence on immunologic binding between solid-phase antibody and IL-3 antigen. There was no evidence of cross-reactivity in the ELISA when excess quantities of human recombinant GM-CSF, M-CSF, G-CSF, TNF, TGFβ, gamma or alpha IFN, c-kit ligand, or erythropoietin were tested in the ELISA. ILs 1, 2, 4, 5, 6, 7, 8, 9, and 10 were all tested in the ELISA and showed no cross-reactivity. To further confirm specificity in the ELISA, we compared standard curves generated in the ELISA with those using the MO-7E IL-3-dependent cell line using an MTT bioassay.12 To exclude any possible effect of GM-CSF in the bioassay system, all test samples were preblocked with anti-GM-CSF antibody. As
shown in Fig 2, levels of recombinant IL-3 assayed in the ELISA (open squares) were comparable with levels detected in the bioassay (triangles) (correlation coefficient = 0.901). We were also able to reproducibly detect IL-3 in supernatants from mitogen- or alloantigen-activated T cells, T-cell clones, or cell lines (MLA-144) known to express IL-3 messenger RNA (data not shown). Finally, in test serum samples that were positive by IL-3 ELISA, anti-IL-3 MoAb was added and samples were re-run in the ELISA. No IL-3 could be detected, further confirming the specificity of the ELISA.

**IL-3 levels in transplant recipients.** IL-3 was not detectable in random serum samples diluted 10-fold from 10 healthy normal volunteers. IL-3 levels were measured in serum samples diluted 10-fold at frequent intervals before, during, and after administration of myeloablative conditioning regimens in 77 patients undergoing various transplant procedures. IL-3 levels were detected in all 40 recipients of non-TCD transplants (autologous and allogeneic) and in 21/57 (37%) of recipients of TCD transplants. A time course of IL-3 levels (open squares) and (ANC) (solid dots) plotted against days posttransplant is shown in Fig 3 for the four different transplant procedures tested. Data points are means of all values observed during 4-day periods (i.e., not just single day peak IL-3 values) for 18 autologous marrow (box A), 10 autologous blood stem cell (box B), 12 allogeneic marrow (box C) and 37 allogeneic TCD marrow transplants (box D). All BM or stem cell infusions occurred on day 0. As shown in Fig 3, IL-3 levels were undetectable before start of myeloablative regimens, i.e., before day −6, in all patients. During administration of myeloablative-conditioning regimens as ANC was beginning to decline rapidly to less than 1,000/μL, IL-3 levels were usually undetectable. However, 22% of samples from autologous transplant recipients tested before day 0 were positive. As ANC decreased to less than 100 μL, usually...
between day +2 to day +10 following BM/stem cell infusion, IL-3 levels peaked. Mean 4-day values ranged between 1,500 to 3,500 pg/mL IL-3 at day +6 although some single day peak values were higher. As neutrophil counts increased to ANC greater than 500/μL, following transplantation, IL-3 levels again declined rapidly and became undetectable in most patients as ANC increased to greater than 1,000/μL by the third week posttransplant.

The relationship between ANC and IL-3 levels for 40 non-TCD transplant recipients is shown in Fig 4. A strong inverse relationship was observed between the circulating IL-3 level and the ANC for TCD BMT recipients (r = -0.897) and non-TCD BMT recipients (r = -0.796).

Single day peak IL-3 levels (shaded bars) and mean day 0 through 14 IL-3 levels (solid bars) observed for each of the four major transplant types and TCD subgroup types is shown in Fig 5. Between day 0 to 14, during optimal release time of IL-3, 137 samples were collected in non-TCD recipients and 117 samples were collected in TCD recipients. Of these, 36/60 (60%) of autologous BMT, 20/33 (61%) of autologous PBSC transplants and 40/44 (91%) of unmodified allogeneic marrow transplant recipient samples had detectable IL-3 levels. In contrast, only 34/117 or 29% of TCD recipient samples had detectable serum IL-3 levels between days 0 and 14. No significant differences between mean single day peak IL-3 levels or average day 0 through 14 IL-3 levels were observed between autologous BMT or unmodified allogeneic BMT recipients (P >

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![Fig 3](image-url) 
**Fig 3.** Timed course of IL-3 levels and ANC for four different transplant types. (e) ANC per microliter. (f) IL-3 levels in picograms per milliliter. Day 0 (arrow) is the day of the infusion of marrow or blood stem cells. Solid bar indicates period of preparative regimen administration. IL-3 levels are averages of 4-day values for each data point representing 18 autologous marrow transplants (A), 10 PBSC transplants (B), 12 unmodified allogeneic marrow transplants (C), and 37 TCD transplant recipients (D).

![Fig 4](image-url) 
**Fig 4.** Correlation analysis of endogenous serum IL-3 levels posttransplant versus ANC for 40 non-TCD recipients (r = -0.796) (light line) and 37 TCD recipients (r = -0.897) (heavy line).

![Fig 5](image-url) 
**Fig 5.** Single-day peak IL-3 levels (■) and mean day 0-14 IL-3 levels (□) for 18 autologous BMT (auto), 12 unmodified allogeneic (allo), 10 PBSC and 37 TCD transplant recipients. Fifteen related and 10 unrelated HLA-identical recipients received a 2.5 to 3.0 log TCD marrow by soybean lecithin agglutination and E-rosetting (SBA−E−). Twelve other related recipients received a 2.0 to 2.5 log TCD soybean lecithin agglutinin and CD5/8 MoAb-coated flasks (SBA−CD5/8−). Values represent mean ± SEM for peak or day 0 through 14 IL-3 levels.
were not significantly different when compared with TCD day 0 through 14 levels (980 pg/mL) were significantly lower in unmodified allogeneic marrow recipients when compared with either autologous marrow peak or day 0 through 14 IL-3 levels. The lowest mean peak IL-3 levels and day 0 through 14 average IL-3 levels were observed in individuals receiving a 2.5 to 3.0 log TCD related donor transplant using the soybean lectin agglutinin E-rosetting method (Fig 5). Although there was a trend for lower peak and day 0 through 14 IL-3 levels in those patients using the more aggressive TCD method, ie E-rosetting versus the CD58 MoAb method, the differences were not statistically significant (P > .1). A trend for higher peak and day 0 through 14 IL-3 levels was also observed in the matched unrelated BMT group compared with the matched related BMT group even though both groups received the same E-rosetting TCD method (Fig 5).

To examine the effects of ATG and methylprednisolone on IL-3 release, we compared IL-3 levels in 15 patients with matched related donors who all received the same TCD method using soybean agglutination and E-rosetting (SBA-E). ATG was administered between day +5 to day +19 in eight of the SBA-E recipients. In 5 of these 8 patients, 8 of 29 day +5 to day +19 samples (27.5%) contained detectable IL-3 with a peak IL-3 level of 3,200 ± 3,193 pg/mL (mean ±1 SD). In contrast, in only 2 of 7 patients not receiving ATG was there detectable IL-3 in 2 of 16 (12.5%) of day +5 to +19 samples with a peak IL-3 level of 329 ± 520 pg/mL (P < .044).

Finally, of the 28 autologous BMT recipients, 15 received posttransplant growth factors, either GM-CSF or G-CSF, and 13 did not. Of the 13 who did not, 12 were evaluable for time to engraftment as one experienced early death. Of the 15 patients who received growth factors, peak IL-3 levels were 2,737 ± 2,142 pg/mL (mean ± 1 SD) versus 4,773 ± 3,141 pg/mL for the 12 autografted patients who did not receive hematopoietic growth factors (P < .01). The patients who received growth factors engrafted to an ANC ≥500 neutrophils/mm³ in 15.7 ± 3.55 days (mean ± 1 SD), compared with 21.2 ± 8.7 days, for the 12 patients who did not receive growth factors (P = .061).

**DISCUSSION**

IL-3, a multipotential stem cell growth factor, may play an important role in the restoration of normal hematopoiesis and lymphopoiesis following the ablative therapy of BM or blood stem cell transplantation. Assessment of the physiologic role of IL-3 in the engraftment process following transplantation has been hampered by the inability to easily quantify low levels of this growth factor in human serum. To address this question, we developed an ELISA that could detect as little as 78 pg of IL-3 in human serum and various T-cell supernatants. The assay did not cross-react with a variety of cytokines that may be present in the posttransplant period, such as GM-CSF, G-CSF, M-CSF, c-kit ligand, IL-1, IL-2, IL-4 through IL-10, IFN-α or γ, TNF, TGFβ, and erythropoietin. The specificity of the ELISA was established by its strong correlation with functional IL-3 levels assayed in the IL-3-dependent MO-7 E cell line. As has been previously observed with human sera in other immunoassays,13,14 we found it necessary to dilute serum 10-fold to recover greater than 95% of the IL-3 in the sample. Under these conditions, we could reliably detect >78 pg/mL of IL-3, enabling us to monitor various serum samples after a variety of transplant procedures. In positive serum samples, we could block detection of IL-3 by preincubating the serum with the anti-IL-3 MoAb further confirming the specificity of the ELISA.

G-CSF and GM-CSF but not IL-3 has been previously detected in serum samples in the immediate posttransplant period.16,17 In the present study, IL-3 was not detected in the serum of normal volunteers or in transplant recipients before initiation of the myeloablative conditioning regimen when WBC counts were normal. In contrast, a predictable burst of IL-3 (up to 6,000 pg/mL) was almost universally observed when ANC declined to less than 100/μL in the immediate posttransplant period (day 0 through 14). As the ANC increased to greater than 1,000/μL, IL-3 usually became undetectable. Thus, there was a strong inverse correlation between IL-3 levels and ANC in both TCD (r = −.897) and non TCD recipients (r = −.796). A similar observation has recently been reported for serum G-CSF levels in the posttransplant period.18 These findings raise the possibility of a feedback mechanism for release of growth factors following myeloablative therapy.

The ability to detect IL-3 in serum samples and the peak IL-3 levels achieved posttransplant during the optimal detection period, ie, in the first 14 days posttransplant, varied between different transplant regimens. That is, 60% to 90% serum samples from the recipients of autologous and conventional allogeneic marrow transplants had detectable IL-3 levels in the immediate posttransplant period. In contrast, only about 30% of samples from TCD transplant recipients had detectable IL-3 levels, and peak levels of IL-3 in the TCD recipient were significantly lower than peak levels in patients undergoing either conventional allogeneic or autologous marrow transplants. Furthermore, in patients undergoing a more vigorous TCD treatment (mean 2.5 v 2.0 logs), a strong trend for lower peak IL-3 levels was observed. Collectively, these findings suggest that optimal release of IL-3 is influenced by T-cell content or function of allografts. The suboptimal IL-3 production in TCD transplants noted here may help explain in part the increased risk of graft failure in these patients.19 In our study, only one patient failed to engraft. IL-3 could not be detected (at an ANC < 100) in this patient, whereas in all other patients where IL-3 was detected engraftment followed. Further prospective studies will be required to determine whether IL-3 detection is required for engraftment.

Lower peak levels of IL-3 were observed in recipients of PBSC transplants in comparison with autologous marrow transplants, although all PBSC patients exhibited an IL-3
spike at neutrophil nadirs and the frequency of positive samples was similar in both autologous marrow and blood stem cell transplant recipients (61% vs 60%). In PBSC transplants, up to $6 \times 10^6$ blood mononuclear cells, containing up to $4 \times 10^9$ T cells are infused compared with approximately $4 \times 10^9$ T cells in autologous marrow recipients. A larger infusion of T cells in PBSC recipients may serve to downregulate IL-3 release. The more rapid engraftment observed in our PBSC recipients (14 days vs. 19 days in autologous or allogeneic marrow recipients) may serve to downregulate IL-3 release through a feedback mechanism. This observation also suggests that peak IL-3 levels do not necessarily determine rate of engraftment (defined as time to ANC $\geq 500/\mu L$), because peak IL-3 levels in PBSC recipients were lower than in T-cell replete autologous or allogeneic marrow recipients.

We observed a lower peak level of IL-3 in patients receiving GM-CSF or G-CSF following marrow transplantation and these patients engrafted to ANC greater than $500/\mu m^3$ earlier (15.7 days) than those not receiving growth factors (21.2 days). These observations also appear to support the concept of a strong inverse correlation between IL-3 levels and ANC. It is possible that exogenous growth factors may serve to downregulate release of IL-3 by accelerating engraftment or by effecting release of IL-3 from T cells or other mechanisms. Further controlled studies will be needed to confirm this observation. However, this study shows how exogenous growth factors may affect multiple growth factor loops that in turn may ultimately influence engraftment.

The exact source of the serum IL-3 in the immediate posttransplant period is uncertain. IL-3 is predominantly produced by activated T lymphocytes or natural killer cells and these cells may survive the conditioning regimen. Recent evidence also suggests that thymic epithelial cells or some marrow stromal cells may possibly also be sources of IL-3.2,4 Damage to any or all of these sources in the recipient after massive chemoirradiation, could trigger release of IL-3. Because IL-3 was detected in autologous marrow or stem cell recipients, alloreactivity is apparently not an absolute requirement for release of IL-3. However, IL-3 was more consistently detectable (Fig 5) at neutrophil nadir in conventional allogeneic marrow recipients (despite use of cyclosporin) in comparison with either autologous or T-cell-depleted allogeneic marrow recipients. Furthermore, peak IL-3 levels were higher in unrelated TCD recipients where alloreactivity may be stronger. Thus, alloreactivity through activation of T cells may also contribute to a release of IL-3 and other cytokines.21,22 This observation is consistent with recent reports of in vivo cytokine release observed during a graft-versus-host disease reaction.23-25 ATG also appeared to increase IL-3 release in TCD recipients of match related SBA $+$ E $-$ grafts. We and others have provided evidence for release of growth factors by ATG. Further clarification of the role of graft-versus-host disease (GVHD) reactions, immunosuppressive therapy, and other factors such as infections on the release of IL-3 will require a large prospective study.

In summary, the present study provides evidence for an acute IL-3 burst that occurs in the immediate posttransplant period of most patients undergoing either allogeneic or autologous marrow or blood stem cell transplants. However, this IL-3 burst may be blunted or undetectable in patients undergoing TCD allogeneic marrow transplants. The peak levels of IL-3 detected were reduced in patients undergoing TCD or PBSC transplants and in those receiving GM-CSF or G-CSF and increased in those receiving ATG. The IL-3 ELISA, by providing a rapid sensitive and specific method for following IL-3 levels in serum posttransplant, will allow further clarification of the physiologic role of IL-3 in the engraftment process and may help guide appropriate use of exogenous IL-3 infusions in the transplant setting.

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REFERENCES

Serum interleukin-3 levels following autologous or allogeneic bone marrow transplantation: effects of T-cell depletion, blood stem cell infusion, and hematopoietic growth factor treatment

KF Mangan, MT Mullaney, TD Barrientos and NA Kernan