Soluble CD4, Soluble CD8, Soluble CD25, Lymphopoietic Recovery, and Endogenous Cytokines After High-Dose Chemotherapy and Blood Stem Cell Transplantation

By Anthony D. Ho, Midori Maruyama, Azzam Maghazachi, James R. Mason, Stefan Glück, and Robert E.T. Corrigham

Mononuclear cell preparations from peripheral blood after mobilization with hematopoietic growth factors have been shown to induce accelerated neutrophil and platelet recovery as compared with that induced by autologous bone marrow transplantation after myeloablative chemotherapy. Because these mononuclear cell products contain many immunocompetent cells other than hematopoietic progenitors, these accessory cells might contribute to the rapid immunohematopoietic reconstitution.

We have monitored the concentrations of soluble CD4 (sCD4), sCD8, and sCD25; the recovery of the lymphocyte subsets and of natural killer (NK) cells; and the endogenous levels of granulocyte colony-stimulating factor (G-CSF), interleukin-3 (IL-3), IL-6, and granulocyte-macrophage-CSF (GM-CSF) in 12 patients who underwent high-dose chemotherapy supported by blood stem cells that were obtained by mobilization with chemotherapy and GM-CSF. The concentrations of both G-CSF and IL-6 peaked at 7 days after reinfusion of stem cells, and this transient elevation preceded the increase in the white blood cell count by approximately 5 to 7 days. The levels of sCD4 and sCD8 increased to a maximum on day 21, and the time to peak levels coincided with the maximum increase in white blood cell count, absolute neutrophil count, or lymphocytes. The levels of sCD25 were found to be elevated from day 7 to day 21. Statistically, the increases in sCD4, sCD8, sCD25, G-CSF, and IL-6 were highly significant, whereas there were no significant changes in IL-3 and GM-CSF. A rapid recovery of the NK activity was found in all 8 of the patients who could be monitored for this assay. Therefore, our study suggests that recovery of CD4+ cells, CD8+ cells, and NK activity coincided with that of neutrophils, which is preceded by a marked, but transient, elevation of IL-6 and G-CSF.

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HIGH-DOSE CHEMOTHERAPY supported by peripheral blood (PB) stem cells has been increasingly used for patients with cancer.1,2 There is now little doubt that leukocyte and platelet recovery is more rapid with blood versus bone marrow (BM) autografts, provided that the blood mononuclear cells (MNC) are collected after mobilization with hematopoietic growth factors, chemotherapy, or both. Because the MNC products obtained by leukapheresis procedures contain a large number of immunocompetent cells, it has been suggested that these accessory cells might have therapeutic as well as restorative potential.1

So far, most of the studies have focused mainly on the neutrophil and platelet recovery after blood stem cell transplant, and only a few reports have provided evidence for an accelerated recovery of lymphocytes.3,4 In previous studies, we and others have shown that granulocyte-macrophage colony-stimulating factor (GM-CSF) has the potential to activate T lymphocytes as well as to mobilize hematopoietic progenitor cells.5-7 Therefore, the use of MNC preparations mobilized by GM-CSF as the source of stem cells for hematopoietic support might induce a rapid immune as well as hematopoietic reconstitution. However, reliable measurements of absolute lymphocytes and their function during severe leukopenia and early recovery is frequently not feasible.

Activated T lymphocytes release various molecules including soluble CD4 antigen (sCD4),5-10 sCD8,11-13 and soluble interleukin-2 receptor (sIL-2R) or sCD25.14,15 Elevated levels of sCD4 have been found in patients with diseases associated with activation of T helper cells (eg, Sjogren's syndrome,16 systemic lupus erythematosus,17 or rheumatoid arthritis18) or in malignant hematologic diseases (eg, acute myeloid leukemia10 or progressive B-chronic lymphocytic leukemia19). Similarly, elevated sCD8 and sCD25 levels have been found in patients with viral infections, autoimmune diseases, and various hematologic malignancies.12-14,20 In patients with acute Epstein-Barr virus infection, the serum sCD8 levels correlated with the percentage of activated CD8+ (CD8+/HLA-DR+) cells.12 In hairy cell leukemia, tumor burden has been reported to correlate with the plasma or serum levels of sIL-2R and CD8.15,20 Measurements of concentrations of these T-cell products might serve as an index for activation of the corresponding T lymphocytes.

In this report, we have monitored the levels of soluble antigens CD4, CD8, and CD25 as indicators for lymphopoietic reconstitution after high-dose chemotherapy and blood stem cell support in 12 patients with metastatic breast cancer. Moreover, their relationships to natural killer (NK) cell activity, to recovery of neutrophil and lymphocyte subpopulations, and to endogenous concentrations of granulocyte-CSF (G-CSF), IL-3, IL-6, and GM-CSF are examined. These kinetic data might provide a better insight into the sequence of immunohematopoietic reconstitution after autologous blood stem cell transplantation (ABSCT).

MATERIALS AND METHODS

Patients. Female patients with metastatic breast cancer who underwent high-dose chemotherapy supported by reinfusion of blood
stem cells were monitored in this study. The age of the patients ranged from 18 to 60 years, and performance status had to be less than 2 according to the World Health Organization scale. The protocol was approved by the Institutional Review Board, and all patients gave written informed consent.

Blood stem cells were harvested after induction chemotherapy that consisted of cyclophosphamide at 750 mg/m² administered intravenously (IV), epirubicin at 100 mg/m² IV, and 5-fluorouracil at 750 mg/m² IV (CEF) all on day 1. The chemotherapy cycle was to be repeated every 21 to 28 days, and 2 to 3 cycles were to be administered. Starting on day 2 of each treatment cycle, GM-CSF (Sandoz Canada Inc, Montreal, Canada) at a daily dose of 5 μg/kg was administered subcutaneously until the leukapheresis procedures were completed. The high-dose chemotherapy regimen consisted of carboplatin (200 mg/m²/d), mitoxantrone (12.5 to 17.5 mg/m²/d), and cyclophosphamide (1.5 g/m²/d), all administered IV, and all administered for 4 days from day −5 to day −2. After 1 day of rest, ABSCs were reinfused on day 0. Within 24 hours of reinfusion, GM-CSF was administered daily at a dosage of 5 μg/kg/d by a continuous IV infusion over 6 hours until the absolute neutrophil count (ANC) was greater than 3.0 × 10⁹ for 3 consecutive days.

Leukapheresis, cryopreservation, and reinfusion of stem cells. Peripheral blood progenitor cells were collected as soon as the white blood cell (WBC) counts approached a level of greater than 2.0 × 10⁹/L and when the platelet counts were greater than 50 × 10⁹/L after induction chemotherapy. Four leukaphereses were performed on 4 consecutive days, unless the patient was febrile or a weekend or holiday intervened. Leukaphereses were performed by means of a blood cell separator (CS3000 Plus; Fenwal Laboratories, Deerfield, IL). A total blood volume of 10 L per apheresis was processed at a flow rate of 60 to 70 mL/min. The apheresis product was centrifuged, part of the plasma was removed, and the product was resuspended with minimal essential medium and was supplemented with 20% dimethylsulfoxide. The cells were then frozen to −10°C using a computer-controlled device, were transferred into the liquid phase of nitrogen, and were stored at −196°C. Thawing of the cryopreserved cells was performed by immersing the freezing bag into a 42°C water bath. The cell suspension was immediately injected into a central line. The target was to obtain a minimum of 1 × 10⁹ kg of CD34+ cells or 2 × 10⁹/kg of colony-forming unit granulocyte-macrophage (CFU-GM) for reinfusion.

Clonogenic assay for hematopoietic stem cells. A total of 2 × 10⁶/mL mononuclear cells was mixed with flowcave’s modified Dulbecco’s medium (GIBCO, Grand Island, NY) containing 0.9% methylocellulose, 5 ng/mL each of GM-CSF and IL-3 (Sandoz AG, Basel, Switzerland), 2.5 U/mL erythropoietin (Behringweike AG, Marber, Germany), and 5 × 10⁻⁷ M 2-mercaptoethanol. The cultures were plated in duplicate and were incubated for 14 days at 37°C in 5% CO₂ in a humidified atmosphere. After 14 days, the cultures were scored using an inverted microscope. The granulocyte-macrophage colonies and erythroid bursts were identified by their typical properties.

Plasma levels of sCD4, sCD8, and sCD25. The levels of CD4 or CD8 antigen in plasma were determined by a sandwich enzyme immunoassay (Cellfree CD4/CD8 Test Kits; T Cell Sciences, Cambridge, MA). An anti-CD4 or anti-CD8 monoclonal antibody (MoAb) was first absorbed onto the wells of a polystyrene 96-well microtiter plate; aliquots of sample diluent and of plasma sample were added into each well and were incubated at 37°C. After washing, a horseradish peroxidase-conjugated MoAb directed against a second epitope on the corresponding molecule was added. After further incubation, the wells were washed, and 100 μL of a substrate solution containing O-phenylenediamine was pipetted into each well. After an incubation of 30 minutes at room temperature, the reaction was quenched with a 2 N solution of sulfuric acid, and the absorbance was measured at 490 nm versus substrate blank. A standard curve was prepared using serial dilutions of a reference standard supplied in the corresponding assay kits. The absorbance of the test wells was then compared with the standard curve and was converted to a numerical value. CD4 or CD8 concentrations were expressed in units per milliliter (U/mL): 1,000 U of CD8 was defined as the amount of released CD8 present in 1.0 mL of a reference preparation provided by the supplier. A unit of CD4 is defined as the amount of CD4 found in 10⁹ Jurkat T cells lysed with 1% NP-40.

Similarly, the levels of sCD25 were determined in the plasma samples by a sandwich enzyme immunoassay (Cellfree IL-2 Test Kit, T Cell Sciences). The details have been described elsewhere.

**Plasma levels of hematopoietic growth factors.** All samples were assayed using sandwich enzyme immunoassays (IL-3, IL-6, GM-CSF, and G-CSF: Quantikine; R & D Systems, Minneapolis, MN). The assay protocols as delineated by the supplier were followed. Polystyrene 96-well microtiter plates were coated with MoAbs against the corresponding growth factor. Recombinant human CSFs (rhCSFs) were used as standards in geometric dilution within a range of 15 to 2,000 pg/mL for IL-3, of 3 to 300 pg/mL for IL-6, of 7 to 1,000 pg/mL for GM-CSF, and of 39 to 5,000 pg/mL for G-CSF, respectively (all supplied together with the assay kits). In addition, growth factors from other suppliers have been used to countercheck the quality of the assays: IL-3 and GM-CSF from Immunex (Seattle, WA); IL-6 from Genetics Institute (Cambridge, MA); and G-CSF from Amgen (Thousand Oaks, CA).

The serial dilutions of the rhCSFs and the patient samples were added to the wells and incubated for 2 hours at room temperature. After washing, a polyclonal murine antibody against a second epitope was added and further incubated for 2 hours at room temperature. The antibodies were directly conjugated to horseradish peroxidase. The color-reaction with a solution of tetramethylbenzidine was stopped with 2 N sulfuric acid after incubation for 20 minutes at room temperature in the dark. The optical density of each well was then determined within 30 minutes using a spectrophotometer set to 450 nm.

NK cell activity. Peripheral blood cells of patients were layered over Ficoll-Hypaque (Pharmacia Chemicals, Ontario, Canada) and were then centrifuged for 25 minutes at 700g to remove red blood cells. The erythrocyte-depleted cell preparation was tested for its ability to kill tumor target cells. Target cells include the NK-sensitive K562 (a human myelogenous leukemia cell line), the NK-resistant but lymphokine-activated killer (LAK)-sensitive targets; RAJI (a human Burkitt lymphoma cell line), and EM-3 (a human leukemic cell line). Target cells (1 × 10⁶) were labeled with 100 μCi ⁵¹Cr (sodium chromate; New England Nuclear/ Dupont, Ontario, Canada) for 1 hour at 37°C, washed, and incubated with various numbers of effector cells in triplicate in round-bottomed wells of microtiter plates (Corning Glass Works, Corning, NY) at 1 × 10⁶ target cells per well in a total volume of 200 μL. The plates were centrifuged and then incubated for 4 hours at 37°C in a humidified atmosphere of 5% CO₂. After the incubation period, supernatants were removed from wells and then counted in a Beckman LS6000IC (Beckman Instruments, Fullerton, CA). Percentage of cytotoxicity was calculated according to the following formula:

\[
\frac{\text{Mean cpm released from test wells} - \text{Mean cpm of spontaneous release}}{\text{Mean total cpm} - \text{Mean cpm of spontaneous release}} \times 100.
\]

**Flow cytometry.** Flow cytometry was performed to determine the lymphocyte subpopulations. Leukocytes in whole blood were stained at room temperature with appropriate antibodies for 20 minutes (fluorescein isothiocyanate- or phycoerythrin-labeled MoAbs...
against CD4, CD8, CD20 and CD25, or negative controls). Erythrocytes were lysed, and leucocytes were fixed with lysis reagent supplied by Coulter Electronics (Hialeah, FL) and according to the instructions of the manufacturer. All MoAbs were obtained from Coulter Electronics, unless otherwise noted. Two-color, four-parameter flow cytometric analysis was performed with a Coulter Elite II as previously described.\(^{22}\) Mononuclear cells were gated by forward- and side-scatter signals. At least 30,000 events were gated, acquired, and stored as list-mode data. The stained cell samples were analyzed within 72 hours after preparation. The absolute numbers were calculated by multiplying the corresponding percentages with the absolute number of lymphocytes.

**Statistical analysis.** For statistical analysis, a personal computer program, Testimate (IDT Dataanalyse, Gauting-Munich, Germany), was used. The Friedman analysis\(^ {23}\) was applied to test the differences in levels of sCD4, sCD8, and sCD25 as well as in plasma levels of endogenous CSFs (IL-3, IL-6, GM-CSF, G-CSF) or lymphocyte subpopulations before and after high-dose chemotherapy and ABSCT.

**RESULTS**

**Patients.** A total of 12 patients were included in the present study. Clinical characteristics of the patients are shown in Table 1. All patients were women, and all had metastatic breast cancer (median age, 41.5 years). The pretransplant WBC count was 4.5 \(\pm\) 0.4/\(\mu\)L (mean \(\pm\) SEM), and the lymphocyte count was 0.58 \(\pm\) 0.05/\(\mu\)L.

**Hematopoietic reconstitution.** The recovery of the WBCs after high-dose chemotherapy and ABSCT in the 12 patients is summarized in Fig 1. The median time to a WBC of greater than 1.0 \(\times\) \(10^9\)/L was 12 days (range, 8 to 16 days), to absolute neutrophil count of greater than 0.5 \(\times\) \(10^9\)/L was 13 days (range, 9 to 18 days), and to an unmaintained platelet count of greater than 20 \(\times\) \(10^9\)/L was 10 days (range, 7 to 27 days). The median number of CFU-GM reinfused was 3.9 \(\times\) \(10^5\)/kg of body weight (range, 0.8 to 12.4 \(\times\) \(10^5\)/kg), and the median number of CD34\(^+\) cells reinfused was 8.9 \(\times\) \(10^5\)/kg (range, 1.4 to 13.8 \(\times\) \(10^5\)/kg). The median number of days for GM-CSF administration was 19 days (range, 15 to 23 days). The correlation between CFU-GM or CD34\(^+\) cell dose and hematopoietic recovery has been reported in detail elsewhere.\(^ {22}\)

**Recovery of lymphocyte subpopulations.** Parallel to the recovery of the WBCs and neutrophils, the absolute number of lymphocytes also increased within the same time frame (see Fig 1). The numbers of total lymphocytes, of cells positive for CD4, CD8, CD20, and CD25 before transplantation (baseline) and at the time of maximum increase in WBCs (21 days after ABSCT) are shown in Fig 2. With the exception of a significant reduction in CD20\(^+\) cells \((P = .0020, \text{Wilcoxon two-sided test}), there were no significant differences in the absolute numbers of cells positive for CD4, CD8, and CD25.

**NK activity of the MNC before and after ABSCT.** Complete serial monitoring of the NK activity could be performed in 8 of 12 patients. The percentage of cytotoxicity activity against K562 cells before induction chemotherapy was 32.8\% \(\pm\) 8.0\% (range, 4.1\% to 52.0\%), and the percentage before high-dose chemotherapy and ABSCT was 26.9\% \(\pm\) 5.3\% (range, <0.1\% to 52.1\%). Whenever possible, the NK activity was measured using the lysis assay. The percentage of K562 cells killed was determined as described in Materials and Methods. Mean value \(\pm\) SEM (error bars) are shown.

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**Table 1. Clinical Characteristics of Patients Studied**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Metastatic Sites</th>
<th>Chemotherapy</th>
<th>Radiation</th>
<th>WBC per nl</th>
<th>Lymphocytes per nl</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>LN</td>
<td>+</td>
<td>–</td>
<td>2.3</td>
<td>0.46</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>LN</td>
<td>+</td>
<td>–</td>
<td>3.7</td>
<td>0.78</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>Lung, bone</td>
<td>+</td>
<td>+</td>
<td>4.6</td>
<td>0.61</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>Bone</td>
<td>–</td>
<td>+</td>
<td>2.9</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>Bone</td>
<td>–</td>
<td>+</td>
<td>4.4</td>
<td>0.86</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>LN</td>
<td>–</td>
<td>–</td>
<td>7.8</td>
<td>0.68</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>LN</td>
<td>+</td>
<td>–</td>
<td>7.2</td>
<td>0.72</td>
</tr>
<tr>
<td>8</td>
<td>54</td>
<td>Lung, bone</td>
<td>+</td>
<td>–</td>
<td>4.3</td>
<td>0.52</td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>LN, bone</td>
<td>+</td>
<td>–</td>
<td>2.6</td>
<td>0.47</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>Lung</td>
<td>+</td>
<td>+</td>
<td>4.1</td>
<td>0.49</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td>Inflammatory</td>
<td>+</td>
<td>–</td>
<td>5.4</td>
<td>0.38</td>
</tr>
<tr>
<td>12</td>
<td>53</td>
<td>LN, lung</td>
<td>+</td>
<td>+</td>
<td>3.9</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Abbreviation: LN, lymph node.
activity was monitored weekly for 6 to 8 weeks after reinfusion of stem cells, but the numbers of MNC available were usually not adequate for the assay until the third week. The recovery of NK activity was also rapid and reached 41.6% ± 8.9% (range, 7.8% to 73.9%) by the third to fourth week after ABSCT. At the same time, cytotoxicity activity against RAJ1 or EM-3 cells was not detected in all 8 patients before ABSCT. In 3 of 8 patients, a slight increase to 7.3%, 8.1%, and 15.0% of cytotoxicity to RAJ1 cells was found, suggesting a generation of LAK-cell activity in a small proportion of the patients after ABSCT and administration of GM-CSF.

**Kinetics of sCD4, sCD8, and sCD25.** The plasma level of sCD4 before induction chemotherapy was 8.7 ± 1.9 U/mL, and before high-dose chemotherapy and ABSCT was 5.5 ± 0.4 U/mL. In our laboratory, both levels were significantly lower than the normal control value of 21.5 ± 1.9 U/mL (range, 10.0 to 36.4 U/mL; n = 11; P < .0001 for both comparisons). There was no significant change in the sCD4 levels within the first 3 weeks after high-dose chemotherapy. The sCD4 level increased significantly in the third week (day 21) after ABSCT to a median of 11.0 ± 0.9 U/mL (range, 7.0 to 15 U/mL; P = .0004, according to Friedmann analysis for matched set of data). The levels of sCD4 returned readily to the pretransplant baseline range in the fourth week. Although the sCD4 levels from weeks 4 to 15 were slightly higher than those before high-dose treatment, the changes were statistically not significant. The results are summarized in Fig 3A.

Before induction chemotherapy with CEF, the plasma level of sCD8 was 308 ± 49 U/mL, and before high-dose chemotherapy and stem cell transplant, it was 258 ± 23 U/mL. The controls in our laboratory showed sCD8 level of 355 ± 23 U/mL (n = 12). The differences between controls and patients in both circumstances were not significant. The
G-CSF (pG/mL)

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limits of detection of the respective assays. The endogenous levels of G-CSF in the 12 patients before induction chemotherapy with CEF and before ABSCT were also in the minimum detectable range (13.9 ± 8.1 pg/mL and 14.5 ± 2.2 pg/mL, respectively) but increased to 690 ± 490 pg/mL (range, 128 to 5,758 pg/mL) in the first week. This increase was statistically significant with a P value of less than .0001 according to Friedmann analysis. Thereafter, endogenous G-CSF levels decreased rapidly and were within baseline range after day 21. The changes in G-CSF levels are summarized in Fig 4A.

Parallel to the increase in G-CSF, the plasma IL-6 levels also increased significantly (P = .0014) from less than 10 pg/mL to 211.1 ± 4.0 pg/mL (range, 3.0 to 42.5 pg/mL) within the first 7 days. After the maximum increase in the first week, endogenous IL-6 decreased rapidly to less than 10 pg/mL and remained at this level. The kinetics of IL-6 levels are shown in Fig 4B. Both GM-CSF and IL-3 were below detection limits before induction chemotherapy, before ABSCT, or after high-dose chemotherapy and ABSCT in all patients.

Relationship between sCD4, sCD8, sCD25, cytokines, and lymphohematopoietic recovery. The time course of the soluble antigens CD4, CD8, and CD25 and of the cytokines G-CSF and IL-6 in relationship to the WBC counts of the 12 patients is shown in Fig 5. There was a synchronous and transient elevation in both G-CSF and IL-6 levels within the first week after ABSCT that preceded the reconstitution of WBCs, whereas the increase in sCD4 and sCD8 levels on day 21 coincided with the maximum increase in WBCs, neutrophils, and lymphocytes. The concentrations of sCD25 remained elevated from days 7 to 21, as compared with baseline, and are probably associated with the administration of GM-CSF, as described previously.6

DISCUSSION

Defects of immune function, as indicated by an imbalance of CD4+ and CD8+ cells, diminished T-cell proliferative

sCD8 levels seemed to decrease slightly in the first 3 weeks after chemotherapy but then increased significantly (P = .0001, Friedmann analysis) to a mean of 638 ± 164 U/mL on day 21. The sCD8 levels remained high until week 10, where it returned to the pretransplant baseline range of the patients. The results are shown in Fig 3B.

The plasma level of sCD25 before induction chemotherapy was 1,945 ± 560 U/mL. After induction chemotherapy and pretransplant, it was 780 ± 117 U/mL. Both were significantly higher than the normal mean of 325 ± 87 U/mL (n = 16; P < .001 for both comparisons). Within the first week after ABSCT, sCD25 levels increased significantly to 2,459 ± 294 U/mL and remained elevated until the fourth week after reinfusion. The sCD25 levels returned to a pretransplant baseline of 750 ± 76 U/mL after week 7, and the kinetic data are shown in Fig 3C.

Endogenous levels of G-CSF, GM-CSF, IL-3, and IL-6. The endogenous levels of GM-CSF, IL-3, and IL-6 before induction chemotherapy and before ABSCT are beneath the limits of detection of the respective assays. The endogenous levels of G-CSF in the 12 patients before induction chemotherapy with CEF and before ABSCT were also in the minimum detectable range (13.9 ± 8.1 pg/mL and 14.5 ± 2.2 pg/mL, respectively) but increased to 690 ± 490 pg/mL (range, 128 to 5,758 pg/mL) in the first week. This increase was statistically significant with a P value of less than .0001 according to Friedmann analysis. Thereafter, endogenous G-CSF levels decreased rapidly and were within baseline range after day 21. The changes in G-CSF levels are summarized in Fig 4A.

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DISCUSSION

Defects of immune function, as indicated by an imbalance of CD4+ and CD8+ cells, diminished T-cell proliferative
responses, depressed IL-2 production, and failure to respond to endogenous IL-2 have all been reported after allogeneic or autologous BM transplantation (ABMT).24,25 We have monitored the levels of sCD4, sCD8, sCD25, the lymphocyte subpopulations, and the NK activities before and after ABCT and have found a rapid reconstitution of the T cells as well as NK cell function that occurred parallel to an accelerated WBC and neutrophil recovery. As previously shown by studies from our group and from others, measurements of the soluble antigens serve as a more sensitive index of activation of the cells expressing the corresponding surface antigen.13,18,20 The sCD4 levels in the patients before induction chemotherapy (CEF), before high-dose chemotherapy and ABCT, and after hematopoietic recovery were significantly lower than that in the normal controls, whereas levels of sCD25 were significantly elevated in the patients under all circumstances. Elevations of sCD25 in various malignancies is well-established and might represent the activation of CD25+ cells as an immune response53-37 or, for lymphoid malignancies such as hairy cell leukemia, as a tumor marker.5 The finding of significantly reduced sCD4 levels in patients with metastatic breast cancer is novel and might reflect the suppression of CD4+ lymphocytes, which could be either associated with advanced disease29,31 or could represent a consequence of previous chemotherapy and/or irradiation therapy.32 Irrespective of the underlying cause, changes in sCD4 or sCD8 have been shown to reflect the activation or suppression of the cells expressing the corresponding antigen.14,20

Analysis of the kinetic data of sCD4 and sCD8 in relationship to endogenous levels of IL-3, IL-6, G-CSF, and GM-CSF showed that the increase in sCD4, sCD8, and in T-lymphocyte subpopulations were preceded by a transient increase in IL-6 and G-CSF levels by 2 weeks, whereas no significant changes in IL-3 or GM-CSF levels were found. Similar elevation of endogenous G-CSF levels has been reported by other investigators after ABCT or ABMT. Cairo et al38 reported a significant increase in G-CSF production around day 5 after ABMT, whereas Kawano et al14 found an increase of G-CSF immediately after graft infusion but no significant changes in IL-3 or GM-CSF levels.34 We found that GM-CSF levels were beneath detectable limits at all times despite administration of GM-CSF after ABCT. In our study, blood samples for soluble antigens and endogenous cytokines were collected at 6 AM, and GM-CSF was administered as a 6-hour continuous infusion between 2 PM to 8 PM. Other investigators have also reported failure to detect significant increases in endogenous GM-CSF levels after ABMT.34-36

Reports on endogenous IL-3 levels after high-dose chemotherapy and ABMT or ABCT are contradictory. Whereas Kawan et al14 and our present report showed no significant change in IL-3 levels, Mangan et al7 detected a burst of IL-3 (peak levels, 1,500 to 6,000 pg/mL) in the immediate posttransplant period between day 0 and day 14.27 Furthermore, a lower percentage of T-cell-depleted transplant recipients had detectable IL-3 levels, and lower peak levels of IL-3 were observed in 60% of the recipients of ABCT. They suggested that a larger infusion of T cells in ABCT recipients might downregulate IL-3 release, and the more rapid engraftment observed in ABCT recipients might also downregulate IL-3 release through a feedback mechanism. At present, we have no satisfactory explanation for the lack of detection of IL-3 in our patients, but the extensive amount of T cells in our MNC preparations stimulated by GM-CSF might suppress IL-3 release, as suggested by Mangan et al.37 Because the levels of these growth factors were determined weekly, we could have missed a very early surge in IL-3 or GM-CSF. However, in 2 of the patients, we monitored the G-CSF, GM-CSF, IL-3, and IL-6 levels every other day for the first 14 days after ABCT, and we noted a surge in G-CSF and IL-6 levels from days 2 to 12. The peak values were within the range measured at day 7, and there were no increases in IL-3 or GM-CSF levels during this period. For this reason, we have subsequently monitored these parameters on a weekly basis.

Contrary to our present observation, an increase in IL-6 was not reported by Kawano et al.34 The blood stem cells in their series were collected after chemotherapy without the addition of cytokines, whereas our patients all received GM-CSF after induction chemotherapy. Rabinowitz et al38 also reported an increase in IL-6 levels after ABCT. Based on a multivariate regression analysis comparing the number of platelet transfusions required with levels of tumor necrosis factor, IL-6, and macrophage-CSF (M-CSF), they concluded that high M-CSF and IL-6 levels on day +12 after ABMT correlated with an increased demand for platelet transfusions and increased toxicity.38 All our patients have recovered from ABCT without major nonhematologic toxicity, and the platelet recovery has been extremely rapid. In previous studies, we have shown both in vitro and in vivo that GM-CSF activates T lymphocytes.57 IL-6 is produced by lymphocytes, monocytes, and mesenchymal stroma cells, and it is likely that MNC preparations mobilized by chemotherapy and GM-CSF contain more activated lymphocytes that produce IL-6 after PBSC and, thus, induce a more rapid reconstitution of platelets. The data from our present study support the notion that there is a fast recovery of lymphocyte counts, in addition to accelerated reconstitution of neutrophil and platelet counts after ABCT. Although the absolute numbers of lymphocytes, CD4+ cells, and CD8+ cells were relatively low after ABCT, the baseline ranges before high-dose chemotherapy were already low and were reached within 3 weeks. In agreement with other studies on lymphocyte recovery after ABCT,3,35,37,38 we also found a tendency for the CD4+ cells to decrease and for the CD8+ cells to increase after ABCT, resulting in a decrease in CD4/CD8 ratio. However, the differences were statistically not significant. Henon et al found a severe imbalance in CD4/CD8 ratio of 0.3 ± 0.2 but found a normal number of B cells after ABCT, whereas Kiesel et al29 reported a CD4/CD8 ratio of slightly less than 1.0 in most patients. We found a moderate (but not significant) decrease of numbers of CD4+ cells and of CD4/CD8 ratio among our patients. In contrast to the studies reported by others,3,35,27,38 the patients examined in the present investigation were homogeneous in their primary tumor diagnosis.
NK cells in patients receiving ABSCT may facilitate the rapid reconstitution of NK activity after ABSCT suggests that the phenomenon of rapid lymphocyte recovery is universally observed in patients undergoing ABSCT.

MNC preparations, obtained by leukapheresis after mobilization regimens, contain numerous immunocompetent cells, especially T helper cells (Ho et al, unpublished results). It has been suggested that such accessory cells might have therapeutic as well as reconstitutive potential and might be responsible for the accelerated neutrophil and platelet recovery after high-dose chemotherapy. This notion is supported by our present study and by a few other comparative studies which show that immunologic as well as hematologic reconstitution is accelerated with the use of PB-versus BM-derived stem cells. However, because we have no comparative control data from patients who received mononuclear cell preparations alone without GM-CSF, the possibility cannot be excluded that the present data on immune reconstitution are associated with GM-CSF rather than with PB-derived stem cells. The results might eventually be different in patients receiving ABSCT without GM-CSF.

NK cells are lymphoid cells with spontaneous cytotoxic activity against a variety of tumor cell lines or primary and metastatic tumor cells. Animal models and clinical observations have demonstrated their vital role in the immune surveillance mechanism. NK cells were found to control established and induced metastases in animal models. A strong correlation was observed between low levels of NK cytotoxicity and diminished time of manifestation of metastatic disease or decreased survival in patients with solid tumors. A rapid reconstitution of NK activity after ABSCT suggests that the immunocompetent cells in the MNC preparations might possess therapeutic potential. The fast recovery of NK cells in patients receiving ABSCT may facilitate the destruction of the remaining tumor cells that may be chemoresistant. NK or IL-2-activated NK (LAK) cells are potent effectors in patients receiving ABSCT without GM-CSF.

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Soluble CD4, soluble CD8, soluble CD25, lymphopoietic recovery, and endogenous cytokines after high-dose chemotherapy and blood stem cell transplantation

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