Autologous Stem Cell Transplantation: Release of Early and Late Acting Growth Factors Relates With Hematopoietic Ablation and Recovery

By Ugo Tasta, Robert Martucci, Sergio Rutella, Giovanni Scambia, Simona Sica, P. Benedetti Panici, Luca Pierelli, Giacomo Menichella, Giuseppe Leone, Salvatore Mancuso, and Cesare Peschle

We have monitored the serum concentrations of hematopoietic growth factors (HGFs); ie, stem cell factor (SCF), leukemia inhibitory factor (LIF), interleukin-3 (IL-3), IL-6, IL-8, and granulocyte colony-stimulating factor (G-CSF) in 15 lymphoma/leukemia and 6 ovarian cancer patients undergoing autologous bone marrow (BM) or peripheral blood (PB) stem cell transplantation (SCT). Thus, the analysis was performed during and after high-dose chemotherapy (from day –6 to day –1), at the time of SCT (day 0), and thereafter (through day +17). Despite the heterogeneity of these patients and their conditioning regimens, a consistent kinetic pattern was observed for all analyzed cytokines. Particularly, (1) SCF serum concentration did not significantly fluctuate. (2) High levels of LIF (<250 to 450 pg/mL) before chemotherapy rapidly declined to markedly lower concentrations (<10 ng/mL) starting from day –1 through day +17; (3) conversely, IL-3 level was low before treatment, sharply increased during chemotherapy, and rapidly returned to baseline level after SCT. Hypothetically, the sharp LIF decrease and IL-3 increase during chemotherapy may underlie the induction of stem cell cycling and differentiation caused by hematopoietic ablation. Furthermore, (4) IL-6 concentration was low before and immediately after chemotherapy, but increased starting from day +5, peaked at day +6 through 9, and then declined to baseline level from day +10 onward; (5) a strictly similar pattern was consistently observed for both G-CSF and IL-8 levels, in agreement with our previous studies. It is relevant that peak IL-6, G-CSF, and IL-8 concentrations were directly correlated to peak neutrophil numbers in the recovery phase, thus suggesting an important role for these cytokines in granulocyte rescue; in line with this interpretation, hematologic patients undergoing PBSCT (10 of 15) exhibited higher peaks of IL-6, G-CSF, and IL-8 and a more pronounced increase of neutrophil/platelet number than did hematologic cases undergoing BMST (5 of 15). Altogether, these studies indicate a coordinate pattern of cytokine release during hematopoietic ablation/recovery after chemotherapy and autologous SCT; the fluctuations of IL-8 and IL-3 levels during chemotherapy are seemingly related to stem cell recruitment, whereas the post-SCT increase of IL-6, G-CSF, and IL-8 may underlie the neutrophil recovery.

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Autologous Bone marrow (BM) and peripheral blood (PB) stem cell transplantation (SCT) is widely used in a variety of hematologic malignancies and solid tumors. After high-dose conditioning therapy, the recovery of hematopoiesis is dependent on stem cell self-renewal and differentiation into lineage-committed progenitors, which undergo differentiation and maturation to morphologically recognizable precursors and terminal cells circulating in PB.

SCT is associated with absolute leukopenia before hematopoietic recovery. Clinical complications associated with prolonged cytopenia are bacterial/fungal infections and severe hemorrhage. Administration of recombinant granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) accelerates hematopoietic recovery. Furthermore, treatment with CSF-primed PB progenitor cells further hastens hematopoietic rescue.

Attention has recently focused on the in vivo secretion of hematopoietic growth factors (HGFs) after high-dose chemotherapy and autologous SCT. High concentrations of serum tumor necrosis factor-α (TNF-α) precede major transplant-related complications. Elevated serum interleukin-6 (IL-6) and macrophage colony-stimulating factor (M-CSF) have been reported in patients developing major organ toxicity. Studies on BMST patients have reported an inappropriate serum erythropoietin response to anemia. Elevation of circulating G-CSF level precedes and correlates with BMST engraftment. In this regard, we have reported a coordinate secretion pattern of GM-CSF, G-CSF, and IL-8 in 10 ovarian cancer patients undergoing autologous PBSCT; particularly, the GM-CSF increase preceded the parallel G-CSF and IL-8 release, which in turn correlated with the subsequent neutrophil recovery.

The present study focuses on the production of cytokines, particularly those acting on the early stages of hematopoiesis (stem cell factor [SCF], leukemia inhibitory factor [LIF], IL-3, and IL-6), in 15 leukemia/lymphoma and 6 ovarian cancer patients undergoing PBSCT and BMST after intensive chemotherapy.

MATERIALS AND METHODS

Patients. Fifteen patients with hematologic malignancies were treated in the Department of Hematology, Catholic University (Rome, Italy) (Table 1). Autografting was performed at either complete remission or a stage of "minimal residual disease." Eight patients had non-Hodgkin's malignant lymphoma, 5 acute myeloid leukemia (AML), and 2 Hodgkin's lymphoma. Ten patients received PBSCT and 5 received BMST. The BuCy2 protocol was used as conditioning regimen, as reported elsewhere. Briefly, patients were treated with busulfan (4 mg/d/kg of body weight) on days –6 through –3, with cyclophosphamide (60 mg/d/kg of body weight) on days –2 and –1 and then transplanted with autologous BM or PBSCs on day 0. The methodology for PBSC harvesting has been described.

Six patients with untreated ovarian cancer eligible for chemotherapy were treated in the Department of Gynecology and Obstetrics, Catholic University (Table 2). All patients had histologic evidence of stage III or IV epithelial ovarian cancer (according to the International Federation of Gynecology and Obstetrics) and a residual tumor of greater than 0.5 cm after cytoreductive surgery. The chemotherapy regimens and methods for harvesting PBSCs have been described. Briefly, patients received cisplatin (100 mg/m², intravenously [IV]) on day -4, and carboplatin (1,800 mg/m², IV) as continuous infusion over 24 hours on day -1. All patients had histologic evidence of epithelial ovarian cancer (according to the International Federation of Gynecology and Obstetrics) and a residual tumor of greater than 0.5 cm after cytoreductive surgery.

Cytokine assays. Serum concentrations of HGFs (SCF, LIF, IL-3, IL-6, G-CSF, and IL-8) were evaluated by sensitive and specific immunoassays (R&D System-British Biotechnology, Cowly, Oxford, UK).

The detection thresholds are 5 pg/mL for SCF, LIF, and G-CSF; 10 pg/mL for IL-3 and IL-8; and 0.3 pg/mL for IL-6. In addition to the controls performed by the supplier, further controls were performed to determine assay specificity (ie, absence of cross-reactivity for each assay against a large panel of recombinant cytokines/proteins). Each cytokine level represents the mean value observed in three separate determinations. The intra-assay variability for the various cytokine determinations was 5% to 10%. The interassay variability of plasma cytokine immunoenzymatic analysis was 5% to 20%, using an identical immunoassay batch for each cytokine.

Statistical methods. Statistical comparisons were performed using the Mann Whitney U test for nonparametric samples, Wilcoxon test for paired analysis, or the binomial proportion test. Statistical significance was defined as \( P < .05 \).

RESULTS

Clinical data. Cytokine serum concentrations were evaluated in 15 leukemia/lymphoma and 6 ovarian cancer patients. The 15 hematologic patients (Table 1) underwent an identical chemotherapy regimen (BuCy2 protocol) before transplantation: 10 patients received transplants of autologous PBSCs and the remaining 5 of BMSCs. The 6 ovarian cancer patients (Table 2) received high-dose chemotherapy followed by PBSCT.

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### Table 1. Hematologic Patients: Main Clinical Features, Infused Cells, and PBMC Recovery

<table>
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<tr>
<th>Patient No.</th>
<th>Age (X10^3/kg)</th>
<th>Diagnosis</th>
<th>Origin</th>
<th>MNC (X10^9/kg)</th>
<th>CFU-GM (X10^9/kg)</th>
<th>WBC &gt;1 X 10^9/L (d)</th>
<th>PMNC &gt;0.5 X 10^9/L (d)</th>
<th>Platelets &gt;50 X 10^9/L (d)</th>
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<td>9</td>
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<td>46.1</td>
<td>11</td>
<td>11</td>
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</tbody>
</table>

Abbreviations: NR, not reached; MNC, mononuclear cells; WBC, white blood cells; NHL, HL, non-Hodgkin's, Hodgkin's lymphoma.
PBSC or BMSC infusion data and kinetics of PB granulocyte and platelet recovery after high-dose chemotherapy and SCT are summarized in Tables 1 and 2. Hematopoietic recovery was prompt and sustained in the 10 PBSCT hemato-logic patients (Table 1); the 5 BMSC patients showed delayed recovery of neutrophils (patients no. 11, 12, and 13) and particularly of platelets (patients no. 3, 10, 11, 12, and 13; Table 1). Granulocyte and platelet recovery was slightly more rapid in the ovarian cancer patients than in the leukemia/lymphoma cases undergoing PBSCT (Table 2). Neutro-phenic fever was reported for most patients (average number of days with >38°C, 3.5; range, 0 to 6), but only 5 cases presented clinically and microbiologically documented in-fectious episodes (the cytokine release pattern in these 5 patients was similar to that observed in the remaining cases). All patients survived at least 100 days after transplantation.

Kinetics of SCF, LIF, IL-3, IL-6, G-CSF, and IL-8 serum levels. SCF, LIF, IL-3, IL-6, G-CSF, and IL-8 plasma levels were evaluated in all 21 patients before and after high-dose chemotherapy/SCT.

SCF, LIF, IL-3, and IL-6 serum concentrations were evaluated in a first set of experiments. SCF, a cytokine present at relatively high levels in the serum of normal subjects, exhibited only slight fluctuations before and after SCT (Fig 1, top). A moderate increase at day 0 was present in 8 of 15 hematologic patients (results not shown). Among hematologic patients, AML BMSC versus lymphoma PBSCT showed similar LIF levels (P = .77). Furthermore, no significant difference was found between hematologic patients undergoing PBSCT versus ovarian cancer patients (P = .80).

Plasma LIF concentrations exhibited a unique pattern (Fig 1, middle). Before chemotherapy LIF was present at high level (mean values, 656, 460, and 218 pg/mL in hematologic BMSC, hematologic PBSCT, and gynecologic patients, respectively; normal LIF serum values are <30 pg/mL; results not shown), then LIF values rapidly and gradually declined until day -2 (~10 pg/mL) and stabilized thereafter. Nineteen of the 21 patients showed a marked decrease of LIF concentration (data not shown). No significant difference was found between hematologic PBSCT versus ovarian cancer patients (P = .79) or among hematologic patients undergoing either BMSC or PBSCT (P = .88).

IL-3 serum levels were low before transplantation, but markedly increased during ablative chemotherapy, peaked on day 0, and then progressively returned to baseline levels after SCT (Fig 1, bottom). The initial IL-3 increase was observed for 20 of 21 patients (data not shown). Plasma IL-3 levels were not significantly different in hematologic PBSCT versus ovarian cancer patients (P = .90) or among hematologic patients undergoing either BMSC or PBSCT (P = .77).

Serum IL-6 levels were low before and during chemotherapy (day -6 to 0) and in the first days after SCT (Fig 2, top). A sharp increase was observed at day +6. All 21 pa- tients exhibited this marked increase (data not shown). In hematologic PBSCT cases, markedly elevated levels of IL-6 (ie, >50 pg/mL) were observed only at day +6; in ovarian cancer, high levels were present from day +6 to +9. IL-6 levels were significantly different in hematologic PBSCT versus ovarian cancer patients (P = .02). Furthermore, among hematologic patients, those undergoing PBSCT exhibited higher IL-6 levels than those undergoing BMSC (P = .05).

We also evaluated the plasma concentrations of G-CSF and IL-8. In agreement with our previous report, all patients exhibited a pronounced increase of both G-CSF and IL-8 levels at day +6 (Fig 2, middle and bottom), ie, 5 to 6 days before neutrophil recovery. As for IL-6, the G-CSF increase is more prolonged in the ovarian cancer than in the lymphoma PBSCT group (day +4 to +11 values, P = .003): in the latter patients, G-CSF peaks occurred at day +6, whereas in the former group, G-CSF peaked at days +6 to +9. However, in these two groups of PBSCT patients, IL-8 exhibited similar kinetics and values (P = .89). Furthermore, among hematologic patients, those undergoing PBSCT showed significantly higher levels of both G-CSF (P = .05) and IL-8 (P = .027) than those undergoing BMSC.

Correlation between cytokine production, neutrophil/ platelet decrease, and recovery. We first evaluated a possi-
**EARLY AND LATE ACTING CYTOKINES**

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**Fig 2.** Kinetics of IL-6, G-CSF, and IL-8 serum concentrations in 10 lymphoma, 5 leukemia, and 6 ovarian cancer patients undergoing SCT after high-dose chemotherapy (see legend to Fig 1). Mean ± SEM values are presented.

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The correlation between cytokine production and neutrophil and platelet recovery was also evaluated. Neutrophil and platelet kinetics are shown in Fig 3. All gynecologic cases and the hematologic patients undergoing PBSCT showed a neutrophil recovery starting from day 11 or 12. A more rapid and sustained neutrophil rescue was observed in the former versus the latter patients ($P = .0004$). No difference was observed between these two groups for the platelet rescue ($P = .60$). In contrast, AML patients undergoing BMSCT showed a delayed and incomplete neutrophil and platelet recovery, in agreement with a previous report. This group significantly differed from PBSCT hematologic patients in both platelet ($P = .004$) and neutrophil recovery ($P = .003$).

In the two groups of patients undergoing PBSCT, peak levels of IL-6, G-CSF, and IL-8 (but not of IL-3) strictly correlated with peak numbers of neutrophils and platelets after SCT (except for the lack of significant correlation between G-CSF and platelet values in gynecologic cases; Table 4).

Hematologic patients undergoing BMSCT exhibited a very delayed platelet recovery and an incomplete neutrophil recovery; the latter parameter did not correlate with the cytokine peaks (Table 4).

**DISCUSSION**

Hematopoiesis is a multistep cell proliferation and differentiation process that is sustained by a pool of hematopoietic stem cells (HSCs). HSCs can self-renew and differentiate into hematopoietic progenitor cells (HPCs), which are committed to a specific lineage(s). HPCs are functionally defined as colony-forming units (CFUs) or burst-forming units (BFUs), i.e., HPCs of the erythroid (BFU-E, CFU-E), the granulomonocytic (CFU-GM, -G, -M), and the megakaryocytic (BFU-MK, CFU-MK) series, as well as multipotent CFU for the GM, E, and MK lineages (CFU-GEHM). In BM, HPCs differentiate into morphologically recognizable precursors that mature to terminal elements circulating in PB.

HGFs control the survival, proliferation, and differentiation quantitative correlation in the three different groups of patients (i.e., hematologic BMSCT, hematologic PBSCT, and ovarian cancer PBSCT) for peak production of IL-3, IL-6, G-CSF, and IL-8 (Table 3). Statistical analysis indicated that (1) IL-3 production does not correlate with release of other cytokines in any of the three groups; and (2) a highly significant direct correlation is present between the levels of IL-6, IL-8, and G-CSF in both hematologic and gynecologic patients undergoing PBSCT. These findings suggest that similar mechanisms may underlie the coordinated production of these cytokines in the analyzed subjects.

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Neutrophil and platelet kinetics are shown in Fig 3. All gynecologic cases and the hematologic patients undergoing PBSCT showed a neutrophil and platelet recovery starting from day 11 or 12. A more rapid and sustained neutrophil rescue was observed in the former versus the latter patients ($P = .0004$). No difference was observed between these two groups for the platelet rescue ($P = .60$). In contrast, AML patients undergoing BMSCT showed a delayed and incomplete neutrophil and platelet recovery, in agreement with a previous report. This group significantly differed from PBSCT hematologic patients in both platelet ($P = .004$) and neutrophil recovery ($P = .003$).

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distal differentiation stage. Erythropoietin, G-CSF, defect of SCF underlies the HSC defect in WW or eages, respectively.4°442

throid, granulocytic, eosinophilic, and monocytic CFU lin-
M-CSF are largely specific for end-stage HPCs of the ery-
the early progenitor pool, ie, CFU-GEMM, BFU-E, CFU-
GM, and early megakaryocytic progenitors. GM-CSF exerts
mice, respectively, thereby suggesting that SCF is one of the
HGFs, particularly IL-3. These early acting GFs include
HGF, ET3 ligand, seemingly acts on primitive hematopoi-
ectic cells.50

In enriched HPC culture, diverse GFs exert little activity
themselves, but potentiate the stimulatory activity of other
HGFs, particularly early acting ones, in a group of
21 leukemia/lymphoma or ovarian cancer cases undergoing
PBSCT or BMSCT. It is noteworthy that, despite the hetero-
geneity of this group of patients and their conditioning regi-
mens, we observed consistent patterns of cytokine release,
particularly (1) a decrease of LIF level early after chemother-
apy precedes a significant increase of IL-3 concentration
through

We previously reported a coordinate release of selected cytokines after chemotherapy and PBSCT in 10 ovarian can-
Address correspondence to...in vivo mechanisms involved in the control of hematopoiesis.51 The serum cytokine con-
centration is dependent not only on the amount of cytokine produced and secreted, but also on the amount bound to
cells and extracellular matrix.51 Furthermore, serum cytokine
levels should be related to other variables that participate in
determining the final biologic outcome (eg, presence of free and
bound cytokine forms and circulating cytokine recep-
tors). Despite these technical limitations, measurement of
circulating HGFs has significantly contributed to the under-
standing of the physiopathology of several infectious and
hematologic diseases.51

We have now investigated the release pattern of a variety of
HGFs, particularly early acting ones, in a group of 21
leukemia/lymphoma or ovarian cancer cases undergoing
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geneity of this group of patients and their conditioning regi-
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tapy precedes a significant increase of IL-3 concentration
before and early after SCT. (2) The release of G-CSF and

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Abbreviation: NE, not evaluated.

All patients were evaluated for the correlation between cytokine and neutrophil peaks. All 16 patients undergoing PBSCT (including 6 of 16 gynecologic and 10 of 15 hematologic cases) were evaluated for the correlation between cytokine and platelet peaks (the remaining 5 hematologic patients undergoing BMSCT presented a delayed platelet recovery; in these cases, we have only evaluated the correlation between cytokine and neutrophil recovery).
IL-8 occurring at later times after SCT, previously described and confirmed here in a large patient group, is associated with a strikingly parallel increase of IL-6. (3) In PBSCT patients, the coordinate peak of G-CSF/IL-8 secretion is directly correlated with the peak recovery of neutrophils and platelets; (4) both phenomena are more pronounced after PBSCT as compared with BMSCCT.

Despite these similarities, gynecologic patients exhibited higher IL-6 and G-CSF posttransplantation levels than did hematologic patients undergoing PBSCT. This difference may be ascribed to different factors, including differences in conditioning regimens, stromal cell function, and/or diseases. However, a plausible explanation could be represented by the fact that ovarian cancer patients were treated by surgery and not by chemotherapy before SCT, whereas hematologic patients received several cycles of chemotherapy to induce remission before SCT treatment.

The initial LIF decrease and IL-3 increase may represent a mechanism underlying HSC proliferation and differentiation during hematopoietic ablation. Previous studies have shown that elevated levels of LIF are required to maintain embryonic stem cells in an undifferentiated status. As mentioned above, IL-3 acts on the early HPC pool and induces the proliferation of primitive quiescent HPCs generating blast cell colonies. Interestingly, the IL-3 peak precedes the GM-CSF peak (see our previous study on ovarian cancer patients undergoing PBSCT). The IL-3/GM-CSF increase, in turn, precedes the elevation of late-acting HGFs (particularly G-CSF and IL-8). Hypothetically, the release of early acting cytokines may stimulate the production of late acting ones. However, it seems likely that, in the BM microenvironment, complex interactions of cytokines, including IL-1 and TNF-α, govern the sequential HGF release after ablative chemotherapy and HSC transplantation.

In addition to the cytokines investigated here, it will be of interest to measure the level of other cytokines acting on primitive HPCs. Particularly, IL-11 has been shown to stimulate megakaryopoiesis and platelet counts in vivo. The synchronous wave of G-CSF, IL-8, and IL-6 secretion at days +5 through 9 after SCT deserves further discussion. The G-CSF, IL-6, and IL-8 peak levels in different patients directly correlate, thus suggesting that a similar mechanism(s) underlies the production of these cytokines. As suggested above, this mechanism may be in part represented by early IL-3/GM-CSF production. The release of G-CSF/IL-8/IL-6 precedes neutrophil recovery. The correlations between peak values of G-CSF, IL-6, and IL-8 and peak neutrophil number in the recovery period and the basic information about the biologic activity of these cytokines strongly suggest a role for these cytokines in neutrophil rescue.

Our observations in PBSCT cases indicate a direct relationship between the IL-6, G-CSF, and IL-8 increase and the platelet rescue. This finding is in line with the finding that both gynecologic and hematologic patients undergoing PBSCT exhibited higher IL-6, G-CSF, and IL-8 posttransplantation levels and a more rapid platelet recovery than did hematologic cases undergoing BMSCCT. Accordingly, previous studies showed a shorter time for platelet recovery in PBSCT as compared with BMSCCT patients.

Although the relationship between the G-CSF/IL-8 increase and the platelet rescue needs further analysis, the role of IL-6 in megakaryocytopoiesis is well established. In vitro, this cytokine stimulates megakaryocyte maturation, as shown by increased megakaryocyte size and development of polyploidy. Furthermore, in vivo studies in rodents and primates have shown that injection of recombinant human IL-6 leads to a twofold to eightfold elevation of platelet counts. Finally, serum IL-6 levels are significantly elevated in patients with reactive thrombocytosis. These findings have led to the speculation that IL-6 and thrombopoietin are identical proteins. The present study supports the hypothesis that IL-6 plays an important role in megakaryocytopoiesis during hematopoietic recovery after ablative therapy and SCT.

In conclusion, this study shows a coordinate pattern of cytokine release during hematopoietic ablation/recovery after chemotherapy and autologous SCT; the fluctuations of LIF and IL-3 levels during chemotherapy are seemingly related to stem cell recruitment, whereas the post-SCT increase of IL-6, G-CSF, and IL-8 may underlie the neutrophil recovery.

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