

Volume 12, issue 2, June 2001 | Recherches

Hematopoietic growth factors in patients receiving intensive chemotherapy for malignant disorders: studies of granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3) and Flt-3 ligand (Flt3L)

Abstract

The levels of hematopoietic growth factors in patients receiving intensive chemotherapy for malignant disorders were investigated using a variety of approaches. Firstly, serum levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF and Flt3-ligand (Flt3L) were examined in acute leukemia patients with treatment-induced cytopenia and complicating bacterial infections. Increased serum levels of both G-CSF and Flt3-ligand (Flt3L) were detected when these patients developed therapy-induced leukopenia, whereas GM-CSF levels were low or undetectable. Development of complicating bacterial infections then increased the serum levels of both G- and GM-CSF, and the Flt3L levels remained high during the infections. Secondly, release of growth factors was characterized for clonogenic T cells that remained in the circulation of acute leukemia patients with chemotherapy-induced cytopenia. CD4+ and CD8+ T cells from these patients released high levels of GM-CSF, relatively low levels of IL-3 secretion having been detected, and only a minority of the clones released detectable amounts of Flt3L. Thus, circulating T cells may contribute to the high systemic growth factor levels in cytopenic patients. Thirdly, plasma levels of GM-CSF and interleukin-3 (IL-3) were examined in patients with malignant disorders who received chemotherapy plus G-CSF for stem cell mobilization. Increased levels of GM-CSF and Flt3L were detected both in the patients' plasma and in the stem cell grafts. Despite the increased growth factor levels in neutropenic patients with complicating infections, the occurrence of febrile neutropenia did not have a major impact on normal hematopoietic reconstitution (i.e. duration of treatment-induced neutropenia) after intensive chemotherapy for acute myelogenous leukemia.

Full text

INTRODUCTION

Hematopoiesis is regulated by a wide range of soluble mediators that interact through the local cytokine network of the bone marrow microenvironment [1, 2]. This local network regulates proliferation and differentiation of normal progenitor cells into mature forms [1], and is also important for the regulation of proliferation, differentiation and survival in leukemic hematopoiesis [2]. The mediators granulocyte-macrophage colony-stimulating factor (GM-CSF), G-

CSF, interleukin-3 (IL-3) and Flt3-ligand (Flt3L) are all important regulators of both normal as well as leukemic hematopoiesis [1-6].

Intensive chemotherapy for acute myelogenous leukemia (AML) results in a disease-free survival of less than 50%, but for subsets of patients this prognosis can be improved by allogeneic bone marrow transplantation (BMT) [7]. Several therapeutic alternatives, with lower treatment-related mortality than allo-BMT, are now considered, including immunotherapy, autologous stem cell transplantation and induction of differentiation and apoptosis [8-10]. Complete hematological remission without previous chemotherapy has been reported in AML patients receiving only antibiotics plus cytokine/growth factor therapy during complicating infections [11-14], and these observations suggest that induction of cytokine cascades may be useful as part of new therapeutic approaches. Cytokine therapy may also be tried in order to enhance T cell reconstitution [15] and antileukemic immune reactivity after chemotherapy [8], or to eliminate contaminating AML cells from autografts, thereby reducing the risk of posttransplant relapse [9].

Local cytokine concentrations in the bone marrow are probably influenced by variations in systemic growth factor levels caused by their release from distant compartments. Both GM-CSF, G-CSF, IL-3 and Flt3L can be released by cells outside the bone marrow compartment [2-6], and we have therefore characterized (i) variations in serum/plasma levels of these growth factors in patients receiving intensive chemotherapy and patients undergoing autologous peripheral blood stem cell (PBSC) harvesting, and (ii) the release of hematopoietic growth factors (GM-CSF, IL-3, Flt3L) by clonogenic T lymphocytes that remain in the circulation during severe, chemotherapy-induced leukopenia.

PATIENTS, MATERIALS AND METHODS

Acute leukemia patients

The local ethics committee approved all studies, and blood samples were collected after informed consent.

T lymphocyte donors. T cell clones (TCC) were derived from five patients receiving intensive cytarabine-based chemotherapy for acute leukemia (Table 1). All five patients developed treatment-induced leukopenia.

The AML blast donor. As accessory cells during T cell activation were used AML blasts derived from a 63 year-old female with AML-M5, secondary to previous chemotherapy. The blasts had multiple chromosomal abnormalities and expressed the membrane molecule phenotype $CD13^{-}CD14^{+}CD15^{+}CD33^{+}$ $CD34^{-}$ $CD45^{+}CD58^{+}CD80^{+}CD86^{+}CD95^{+}$ (+ defined as > 20% positive cells judged by flow cytometry). Previous studies had demonstrated that these cells were effective as accessory cells during T cell activation [16].

Studies of cytokine serum levels. During a period of 18 months, serum samples were collected three times a week from all patients receiving intensive chemotherapy (*i.e.* chemotherapy regimens expected to give at least 7 days of cytopenia) for acute leukemia at our institution. Clinical evaluation, peripheral blood counts and serum CRP levels were recorded at the same time. Samples were collected before and following postremission consolidation courses except for patients who were to receive the consolidation therapy at another institution. For these last patients, serum samples were collected following induction therapy, and bone marrow examination 14 days after the start of therapy confirmed that the patients had no detectable residual disease. The samples were stored frozen at - 70° C until thawed and analyzed.

The patients who developed bacterial infections were included in the present study. To be classified as a bacterial infection all the following criteria had to be fulfilled: (i) fever > 38° C lasting for at least 4 hours; (ii) serum C reactive protein > 100 mg/l; (iii) bacterial growth in blood cultures or local signs of bacterial infection. Nineteen episodes of bacterial infection were studied, 10 of these being septicemia. Samples were selected for analysis according to the following criteria:

- pretherapy: samples collected immediately before the start of consolidation therapy;

- cytopenia: the last sample collected before development of infection (less than 3 days before fever), at this time the patients were afebrile with no clinical signs of infection and with normal serum CRP levels. We also included two consolidation courses that were not complicated with febrile neutropenia, in these cases the samples collected after 7 days of neutropenia were selected;

- infections: the first sample collected (within 3 days) after development of fever while the patients still fulfilled the criteria for bacterial infection;

- antibiotic therapy: the first sample collected after clinical improvement (afebrile patient, serum CRP < 50 mg/l), but the patient still being cytopenic (see below).

All patients had a central venous (Hickman) catheter, and they all developed therapy-induced cytopenia, with white blood cell (WBC) counts < 0.5×10^9/l and dependency on platelet transfusions for maintenance of peripheral blood platelet counts > 10×10^9/l. The median duration of cytopenia was 21 days (range 12-42 days), and the patients were treated in protective isolation. Patients received antibiotic therapy when they developed fever. They were initially treated with intravenous penicillin + netilmicin (+ metronidazole if an anaerobic infection was suspected). If fever persisted longer than 3-4 days, and a causative organism had not been isolated, empirical therapy was changed to either imipenem, cephtazidime + netilmicin (perianal infections) or vancomycin + netilmicin (skin infections). No patient had documented infections with fungi, *Pneumocystis carinii* or *Clostridium difficile*.

Samples were taken from 15 AML patients and 3 acute lymphoblastic leukemia (ALL) patients (6 females and 12 males, age 17-69 years), who were studied over 21 chemotherapy courses. Two patients were only available for a limited number of cytokine studies. The clinical status before chemotherapy was: acute leukemia, 8 courses; first complete remission, 10 courses; and second complete remission, 3 courses. All patients received intensive chemotherapy using the following regimens: (i) mitoxantrone 12 mg/m^2 day 1-3 + cytarabine 200 mg/m^2 day 1-7, 1 course; (ii) daunorubicine 50 mg/m^2 day 1-3 + cytarabine 200 mg/m^2 day 1-7, 5 courses; (iii) amsakrine 150 mg/m^2 day 1-3 + cytarabine 100 mg/m^2 day 1-5 + etoposide 110 mg/m^2 day 1-3, 11 courses (two of these patients did not develop infections); (iv) cytarabine 3 g/m^2 x 2 days 1, 3, 5 + etoposide 100 mg/m^2 day 1 and 5, 2 courses; (v) daunorubicine 50 mg/m^2 day 1-3 + cytarabine 200 mg/m^2 + thioguanine 150 mg/m^2 day 1-5, 1 course; (vi) cytarabine 1 g/m^2 day 1-5 + mitoxantrone 12 mg/m^2 day 1-3, 1 course. Serum samples were also collected from healthy controls matched for age and sex.

Patients undergoing PBSC harvesting. Plasma samples were collected during 16 apheresis procedures in 12 consecutive patients (5 patients with multiple

myeloma, 4 patients with non-Hodgkin's lymphoma and 3 patients with sarcoma). Samples from PBSC grafts were available for 14 procedures. Stem cells were mobilized by chemotherapy + G-CSF for all except one patient who received only high-dose G-CSF. The median WBC count in venous blood immediately before harvesting was 20.6 x 10^9/l (range 4.0-74). Mobilized stem cells were collected on a CobeSpectra Cell-Separator (COBE, BCT Inc., Lakewood, CD, USA), using acid citrate dextrose (ACD) for anticoagulation. The stem cells were usually collected from 2.5-3 times the patients total blood volume, but for one apheresis, a larger volume and for two apheresis, smaller volumes were used. All PBSC grafts contained at least 2 x 10^6 $CD34^+$ cells per kg body weight.

Preparation of peripheral blood and graft plasma samples was based on the previous studies by Files *et al.* [17]. To avoid platelet activation during *in vitro* handling of samples, we used an anticoagulation mixture consisting of (i) ACD (derived from Becton Dickinson vacutainer tubes, solution A); (ii) acetylsalicylic acid (ASA; Sigma, UK) dissolved in 0.1 M $NaHCO_3$ and prepared immediately before sample collection; (iii) prostaglandin E_1 (PGE_1, Sigma) dissolved in 96% ethanol and stored at - 70° C until use. The final concentrations of ASA and PGE_1 in samples were 1mM and 1 muM respectively, and the final ethanol concentration was 0.1%. The anticoagulation mixture was prepared less than 30 min prior to sample aspiration. Venous blood samples were collected from the patients immediately before and after stem cell apheresis, by careful aspiration *via* the patient's central venous catheter. Five ml of blood were always aspirated and discarded before sampling into precooled syringes containing 1 ml of ACD, 100 mul ASA and 5 mul PGE_1 solution (total volume 5 ml, blood sample volume 3.9 ml). Graft samples were collected within 40 min after stem cell collection, by careful aspiration into precooled syringes containing 0.5 ml ACD, 50 mul ASA and 2.5 mul PGE_1 (total sample volume 2.5 ml). Venous blood samples from healthy blood donors (matched for age and sex) were collected immediately after blood donation by careful aspiration from the collection device as described above. All samples were thereafter transferred to precooled plastic tubes, rested on ice for 10 min and finally centrifuged at 33,000 g for 20 min at 4° C. The supernatants were then collected and stored at - 70° C until analyzed.

Analysis of treatment-induced neutropenia in AML patients. All adult patients (n = 81; age 15-71 years) receiving intensive (remission induction and/or consolidation therapy) for AML at our institution, during the period 1989-1998,

were included in the study. None of the patients received hematopoietic growth factor therapy.

Reagents for tissue culture

The culture medium was RPMI 1640 with glutamine and HEPES (Gibco; Paisley, UK), to which was added gentamicin 100 mug/ml and 10% inactivated fetal calf serum (BioWhittacker; Walkersville, MA, USA). Recombinant human IL-2 was supplied by PeproTech (Rocky Hill, NJ, USA), and used at 20 ng/ml.

Preparation of AML blasts

AML blasts were prepared from peripheral blood by density gradient separation (Ficoll-Hypaque with specific density 1.077; NyCoMed, Oslo, Norway), and the percentage of leukemic cells in this population exceeded 98% [16].

Preparation of T lymphocyte clones

TCCs were prepared as described in detail previously [16]. Briefly, $CD4^+$ and $CD8^+$ cells were isolated by immunomagnetic bead separation from the peripheral blood of acute leukemia patients with treatment-induced cytopenia (Table 1). The T cells were thereafter seeded into 60-well culture plates (20 mul/well; Nunc, Copenhagen, Denmark) according to a limiting dilution, with each well containing 1×10^4 accessory cells (30 Gy irradiated pooled, allogeneic peripheral blood mononuclear cells; PBMC), 1 mug/ml phytohemagglutinin (PHA HA16; Wellcome, Dartford, UK), and 10 ng/ml of recombinant human IL2 (PeproTech). After 10 days, individual clones were transferred to separate wells of flat-bottomed microtiter plates (Costar 3596, Cambridge, MA, USA; 270 mul/well) and restimulated by coculture with allogeneic PBMC (5×10^4/well), PHA and IL-2. Seven days later, TCC were transferred to 24-well culture plates (Costar 3524; 2 ml medium/well) and restimulated by adding PHA, IL-2 and 5×10^5 allogeneic accessory cells/well. IL-2 was also added 3 days after restimulation. Cultures were split when the cells formed a monolayer. One additional restimulation was performed 1 week later, and 7 days thereafter the TCC were frozen in liquid nitrogen for storage.

Flow cytometric analysis demonstrated that the clones were of the phenotype $CD2^+CD3^+$ $CD56^-$ T cell receptor (TCR) $alphabeta^+gammadelta^-$ and either

$CD4^+CD8^-$ or $CD4^-CD8^+$ [16]. All TCC were derived from cultures seeded with a cell number lower than one clonogenic cell per well [16], and the flow cytometric analysis demonstrated cell distribution curves consistent with monoclonality for all clones and surface markers.

Determination of growth factor concentrations in supernatants and serum

Cytokine release by in vitro*-cultured TCC.* As described in detail previously [16], PHA-stimulated cytokine release was analyzed by incubating 2×10^4 cloned T cells in each well of U-bottomed microtiter plates (Costar 3796), together with PHA, IL-2 and 5×10^4 non-irradiated AML accessory cells/well. Supernatants were harvested after 72 hours.

ELISA assays. All ELISA analysis of serum samples and culture supernatants (Quantikine ELISA; R&D Systems Europe, Abingdon, UK) were performed strictly according to the manufacturer's instructions [16]. Standard curves were constructed using the mean of duplicate determinations, and differences between duplicates were generally < 10% of the mean. The minimal detectable levels were GM-CSF (supernatants) 2.8 pg/ml, GM-CSF high sensitivity (serum/plasma samples) 0.26 pg/ml, G-CSF 0.4 pg/ml, Flt3L 7 pg/ml and IL-3 7.4 pg/ml.

Presentation of the data

For ^{3}H-thymidine incorporation assays, the median counts per minute (cpm) of triplicate cultures were used for all calculations and comparisons. The incremental proliferative response was defined as the response (cpm) in stimulated cultures minus the response in unstimulated controls. Significant proliferation was defined as an incremental response corresponding to > 1,000 cpm and exceeding the negative control by at least 3 standard deviations (SD). The Wilcoxon's test for paired samples and the Wilcoxon's rank sum test were used for statistical comparisons, and for correlation analysis the Kendall's test was used. Differences were regarded as statistically significant when $p < 0.05$ after correction for the number of comparisons.

RESULTS

Serum levels of hematopoietic growth factors in acute leukemia patients receiving intensive chemotherapy; studies of G-CSF, GM-CSF and Flt3L

Serum levels of G-CSF, GM-CSF and Flt3L were determined for 13 matched controls and 13 acute leukemia patients in complete hematological remission who were tested before the start of chemotherapy and after the development of therapy-induced cytopenia (Figure 1). The pretherapy levels did not differ significantly from the levels in the controls for any of the three cytokines, although the Flt3L and G-CSF levels showed a wider variation in patients than in controls. However, when the patients developed therapy-induced cytopenia the serum levels of both Flt3L ($n = 17$, Wilcoxon's test for paired samples, $p < 0.002$), G-CSF ($n = 19$, $p < 0.048$) and GM-CSF ($n = 17$, $p < 0.005$) increased significantly compared with pretherapy levels.

Serum levels of Flt3L, GM-CSF and G-CSF were also examined in cytopenic patients with complicating bacterial infections, and after clinical improvement due to antibiotic therapy (Figure 1). The levels of GM-CSF ($n = 17$; Wilcoxon's test for paired samples, $p < 0.005$) and G-CSF ($n = 19$, $p < 0.002$) increased during infections, before decreased levels were observed during clinical improvement. For Flt3L, the increased levels persisted ($n = 17$; $p < 0.004$ compared with the controls) and did not alter during infection and antibiotic therapy.

Secretion of hematopoietic growth factors by TCC derived from acute leukemia patients with chemotherapy-induced cytopenia; studies of GM-CSF, IL-3 and FLT3L

Secretion of hematopoietic growth factors was investigated for a panel of $CD4^+$ and $CD8^+$ TCC derived from 5 acute leukemia patients with treatment-induced cytopenia. The results for GM-CSF and IL-3 are presented in Figure 2. All TCC showed detectable GM-CSF levels. Although GM-CSF release showed a wide variation for the two T cell subsets, the levels were significantly higher for the $CD4^+$ than the $CD8^+$ clones (Wilcoxon's rank sum test, $p < 0.001$). IL-3 levels were lower than the corresponding GM-CSF levels for all TCC ($p < 0.002$), and significantly higher IL-3 levels were detected for the $CD4^+$ than for the $CD8^+$ clones ($p < 0.001$). In contrast to GM-CSF and IL-3, the majority of clones showed undetecTable Flt3L, and low but detectable levels (range 7.1-15.6 pg/ml) were observed only for 7 $CD4^+$ and 6 $CD8^+$ TCC.

Levels of hematopoietic growth factors in stem cell grafts and venous blood during PBSC harvesting; studies of GM-CSF and Flt3L

The plasma levels of GM-CSF and Flt3L were compared for PBSC grafts and venous blood derived from the patients and from matched healthy controls. Increased levels of both cytokines were then detected in the autografts (Figure 3; Wilcoxon's rank sum test, $p < 0.001$ for both cytokines) compared to normal venous levels. Preharvesting blood levels in patients did not differ significantly from the PBSC levels and were significantly increased compared to the controls both for Flt3L (n = 16, median 168 pg/ml, range 74-410 pg/ml, $p < 0.001$) and GM-CSF (n = 16, median 1.2 pg/ml, range 0.3-6.0 pg/ml, $p < 0.001$). The harvesting procedure had only a minimal influence on venous cytokine levels and did not alter GM-CSF levels significantly, whereas for Flt3L, a small but statistically significantly decrease was observed after harvesting (n = 16, median 139 pg/ml, range 45-315 pg/ml, Wilcoxon's test for paired samples, $p < 0.002$).

Hematopoietic reconstitution after intensive chemotherapy for AML

The duration of treatment-induced leukopenia was determined for 255 chemotherapy courses in 81 AML patients. Median duration of leukopenia for all cycles was 24.0 days, but the chemotherapy regimen had a major impact on the duration of treatment-induced leukopenia and the number of days with febrile leukopenia (Table 2). Furthermore, the duration of leukopenia and the percentage of febrile days did not differ significantly when comparing first and second remission induction with daunorubicin plus cytarabine in 14 patients with leukemia relapse, and no difference was observed between the first and subsequent courses of consolidation therapy with amsakrin + cytarabine + etoposide (22 patients receiving 2 courses) or high-dose cytarabine (11 patients receiving up to four courses).

To investigate whether the frequency of leukopenic days with fever had any effect on hematopoietic reconstitution, we compared the duration of neutropenia ($< 0.5 \times 10^9$/l) for chemotherapy courses with > 50% and < 50% of leukopenic days with fever. However, no significant difference in the total duration of neutropenia was detected between cycles with high and low frequencies of febrile days, neither when comparing the overall results nor when comparing the results for induction courses, consolidation courses or different treatment regimens separately.

DISCUSSION

Intensive chemotherapy resulting in therapy-induced cytopenia is used in the treatment of acute leukemias and as a part of PBSC mobilization regimens [18, 19]. It is well-known that intensive chemotherapy can alter the systemic levels of several cytokines [20], but very few of the previous studies have investigated the levels of hematopoietic growth factors. In the present study, we have therefore characterized how high-dose chemotherapy affects the systemic levels of hematopoietic growth factors.

Increased serum erythropoietin and M-CSF levels have been described for cancer patients with neutropenia [21-24], but only one previous study reported by Cebon *et al.* [24] has investigated serum levels of G-CSF and GM-CSF in patients with therapy-induced neutropenia. However, in contrast to our present study Cebon *et al.* included patients with various malignant disorders, neutropenia was defined as < 1.0×10^9/l, and patients were not followed longitudinally after chemotherapy. These investigators reported that high G-CSF levels were associated with neutropenia and fever. Similarly, our present longitudinal study of severely neutropenic patients with acute leukemia revealed increased G-CSF levels in neutropenic patients and an additional increase during complicating infections. Furthermore, in contrast to Cebon *et al.* we observed increased GM-CSF levels during cytopenia, with an additional increase during infection. Increased GM-CSF levels were also detected during stem cell harvesting.

A recent study described increased serum Flt3L levels after conditioning therapy of patients treated with autotransplantation for malignant lymphoma and multiple myeloma [25]. Our present results demonstrated that increased Flt3L levels are also detected during PBSC harvesting. Serum levels of Flt3L have not been investigated previously in acute leukemia patients, and in our present study we report that these patients also have increased levels during therapy-induced neutropenia.

Growth factor release by clonogenic T cells was investigated in a standardized experimental model. Accessory AML cells were then derived from the same patient, the mitogen PHA was used because it interacts with T cell molecules that also are important in antigen-stimulated activation [26], and IL-2 was added to optimize the activation signal [27]. GM-CSF levels were generally

higher than IL-3 levels, $CD4^+$ TCC showed higher levels of both these growth factors than $CD8^+$ T cells, and Flt3L release was low or undetectable. Since activated T cells are detected in the circulation of acute leukemia patients with treatment-induced cytopenia [28], our present results suggest that T cells contribute to systemic growth factor levels in neutropenic patients.

We regard autotransplantation as experimental AML therapy [9], and for this reason samples from PBSC-transplanted AML patients were not available at our institution. However, our PBSC-mobilized patients received preharvesting chemotherapy to induce disease regression, and stem cell mobilization and harvesting were performed according to standardized procedures. Thus, our results are probably representative also for other patients (*e.g.* AML patients) treated according to the same guidelines. Increased Flt3L and GM-CSF levels were detected both in the patients' blood and PBSC grafts, and the apheresis procedure caused only minimal alterations of the blood levels with a small (but statistically significant) decrease in Flt3L levels after harvesting. Because the growth factor levels may affect the functional phenotype and thereby also the survival of graft-contaminating AML cells, these levels may have an impact on the risk of posttransplant AML relapse that can derive from residual graft disease [29].

To further examine the possible effects of infection-induced cytokine cascades on normal hematopoiesis, we investigated whether a high percentage of leukopenic days with fever was associated with an altered time period until hematopoietic reconstitution after intensive AML therapy. The duration of neutropenia varied between patients, and was, in addition, dependent on the chemotherapy regimen. The younger age (< 60 years, see ref. 7) of patients receiving high-dose Ara-C probably also contribute to the relatively short duration of neutropenia in these patients. However, duration of neutropenia did not differ for patients with high or low percentages of days with febrile neutropenia. These results suggest that the increased systemic growth factor levels induced by complicating infections do not have any major impact on the reconstitution of normal hematopoiesis. It cannot be judged from our present results whether the increased growth factor levels have any effect on residual leukemic disease, but previous case reports suggest that *in vivo* cytokine cascades may affect leukemic hematopoiesis in AML [11-14].

CONCLUSION

Our present results demonstrate that systemic levels of several hematopoietic growth factors are altered in patients receiving intensive chemotherapy. Although some previous case reports suggest that cytokine cascade reactions can mediate antileukemic effects in AML, it is still not known whether these increased levels are clinically important in AML patients receiving intensive chemotherapy.

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Figures

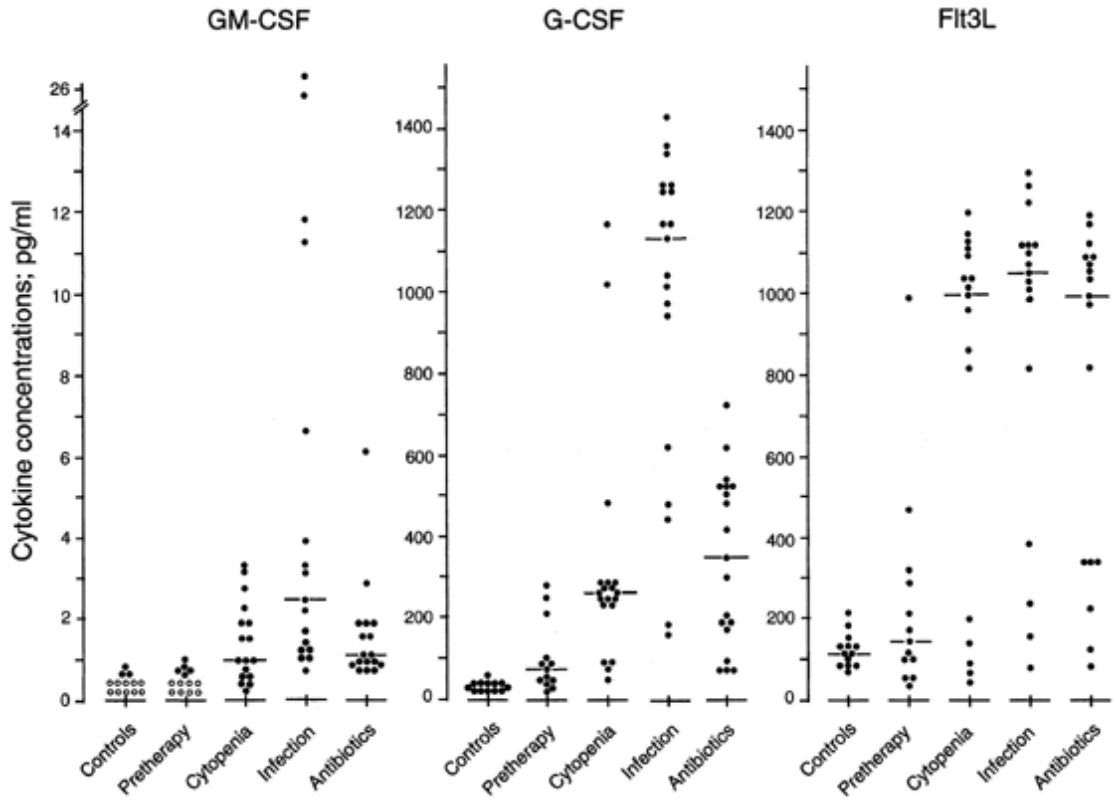


Figure 1

Serum levels of GM-CSF (left), G-CSF (middle) and Flt3L (right) in healthy controls and in acute leukemia patients.

The patients were tested at different times before and following the start of intensive chemotherapy (see bottom of the Figure):

- pretherapy: sample collected immediately before the start of consolidation chemotherapy (n = 13);

- cytopenia: sample collected from patients with chemotherapy-induced cytopenia and no signs of bacterial infection. The Figure presents the data for patients who later were examined during bacterial infection and antibiotic therapy (n = 17 for GM-CSF and Flt3L, n = 19 for G-CSF); these patients then included 11 of the patients tested before the start of consolidation therapy;

- infections: serum levels examined during complicating bacterial infections in patients with therapy-induced cytopenia.

- antibiotics: serum levels examined in cytopenic patients after clinical improvement due to antibiotic therapy.

All concentrations are presented in pg/ml, and the median value is indicated (- ▒ -).

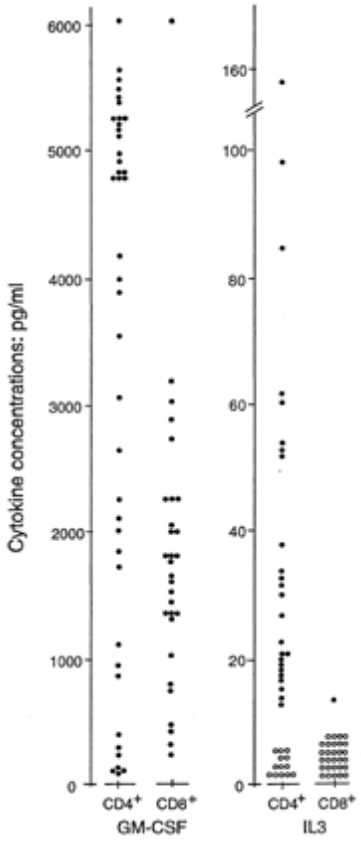


Figure 2

Release of GM-CSF (upper) and IL-3 (bottom) by TCC stimulated with PHA + IL-2 + AML accessory cells.

Cells from 40 (for IL-3 only 38 available TCC) $CD4^+$ and 30 $CD8^+$ clones (all clones of the phenotype $CD2^+$ $CD3^+$ $TCRalphabeta^+gammadelta^-$ and either $CD4^+CD8^-$ or $CD4^-CD8^+$) were activated in the presence of non-irradiated, native AML blasts derived from the same AML patient. The incremental cytokine levels for $CD4^+$ and $CD8^+$ clones are presented separately as indicated in the Figure. UndetecTable cytokine levels are indicated in the Figure (o). Cytokine levels (pg/ml) were determined in the supernatants by ELISA analysis after 72 hours of culture.

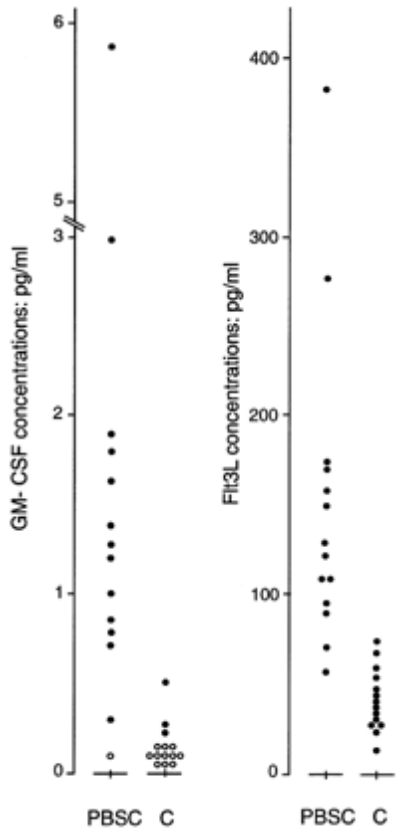


Figure 3

Plasma levels of GM-CSF (upper) and Flt3L (bottom) in venous blood of 14, matched, healthy controls (indicated as C) and in PBSC grafts derived from 14 patients with malignant disorders (indicated as PBSC).

Stem cells were harvested after mobilization with chemotherapy + G-CSF therapy. UndetecTable levels are indicated in the Figure (o).

Table 1
Clinical characteristics of acute leukemia patients used for preparation of T lymphocyte clones

Characteristics	Patient A	B	C	D	E
Status before last chemotherapy course	Untreated AML	AML in complete remission	Untreated ALL-relapse	Untreated AML	Untreated AML
Last chemotherapy course	Ara-C 200 mg/m² days 1-7 daunorubicin 45 mg/m², days 1-3	Ara-C 3 g/m² twice daily, days 1, 3, 5	Ara-C 3 g/m² days 1-4 etoposide 100 mg/m²	Ara-C 200 mg/m² d1-7 daunorubicin 45 mg/m² days 1-3	Ara-C 200 mg/m² days 1-7, daunorubicin 45 mg/m² days 1-3
T cell cloning					
Days after start of cytopenia[1]	11 (23)	7 (13)	9 (15)	8 (26)	10 (21)
Number of CD4⁺/CD8⁺ clones studied	1/2	18/0	1/3	15/23	6/2

[1] Cytopenia defined as leukocytes < 0.5 and/or dependency on thrombocyte transfusions to maintain thrombocyte levels > 10 × 10⁹/l. In parenthesis is given the total duration of cytopenia.

Table 2
Duration of leukopenia in adult patients receiving intensive chemotherapy for AML

Chemotherapy	Number of courses	Duration of neutropenia (days)[1]	Number of days with fever
All chemotherapy courses[2]	255	24.0 ± 10.3	6.7 ± 4.8
Induction *versus* consolidation			
Induction courses resulting in complete remission	70	31.8 ± 12.6	12.7 ± 6.6
All first consolidation courses	70	18.2 ± 9.4	6.6 ± 6.0
Different chemotherapy combinations			
Induction courses			
– Daunorubicine 50 mg/m² days 1-3; cytarabine 200 mg/m² days 1-7	63	31.1 ± 12.1	11.0 ± 8.0
– Mitoxantrone 12 mg/m² days 1-3; cytarabine 200 mg/m² days 1-5	7	26.0 ± 6.2	7.4 ± 5.5
Consolidation courses			
– Amsakrine 150 mg/m² days 1-3 or 1-5; cytarabine 100 mg/m² days 1-5; etoposide 110 mg/m² days 1-3 or 1-5	66	22.9 ± 12.7	4.3 ± 5.0
– Cytarabine 3 g/m² twice daily on days 1, 3 and 5	36	14.4 ± 3.5	2.8 ± 2.6

[1] Duration of leukopenia/fever is presented as the mean duration (days) ± standard deviation.
[2] For induction: duration until reconstitution or defection of leukemia.

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In this issue

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acute lymphoblastic leukemia, SDF-1a, CXCR-4, VLA-4, VLA-5, fibronectin.

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chemotherapy, acute leukemia, G-CSF, GM-CSF, IL-3, Flt3L.

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Sambucol, inflammatory cytokines, IL-1b, TNF-a, IL-6, IL-8.

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interleukin-18, malaria, Plasmodium falciparum, gamma interferon.

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THP-1 cells, macrophages, estrogen receptors, estrogens, monocytes.

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