SERUM LEVELS OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR AND GRANULOCYTE COLONY-STIMULATING FACTOR IN TREATED PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE

Enrico Balleari, Caterina Bason, Giuseppe Visani*, Marco Gobbi, Emanuela Ottaviani*, Riccardo Ghio

Department of Internal Medicine, University of Genoa, Italy; *Institute of Hematology “L. & A. Seràgnoli”, University of Bologna, Italy

ABSTRACT

Background. Despite the fact that granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) are increasingly used in clinical practice, little is known of their endogenous production, especially in myeloproliferative disorders such as chronic myelogenous leukemia (CML).

Methods. In order to define serum levels of GM-CSF and G-CSF in subjects affected by CML, the sera of 17 patients with CML in chronic phase treated either with hydroxyurea or interferon-alpha were tested by specific enzyme immunoassays. Fifteen age- and sex-matched healthy volunteers were used as normal controls.

Results. Eight out of the 17 patients (44%) with CML showed detectable (> 3 pg/mL) serum levels of GM-CSF (range 3.9-55 pg/mL). Detectable levels (> 50 pg/mL) of G-CSF were observed in 9 of these patients (52%) (range 150-2,830 pg/mL). On the contrary, among the normal controls only one had detectable GM-CSF concentrations, and none had detectable G-CSF concentrations. The highest concentrations of both GM-CSF and G-CSF were seen in patients with the highest white blood cell counts, although a linear correlation between the levels of these growth factors and the number of circulating leukocytes was not demonstrated.

Conclusions. Our data indicate that significant amounts of both endogenous GM-CSF and G-CSF are detectable in the serum of a substantial percentage of patients with CML in chronic phase. The pathophysiological meaning of this finding remains to be determined.

Key words: CML, endogenous GM-CSF and G-CSF
Calculating leukocytes in myeloproliferative disorders such as chronic myelogenous leukemia (CML) is largely unknown. Therefore in the present study we investigated the sera of CML patients in chronic phase by means of specific enzyme immunoassays in order to assess the concentrations of both GM-CSF and G-CSF. Furthermore, correlations between these myeloid CSF and blood counts were evaluated in order to speculate on their possible role in the expansion of the neoplastic clone in this form of leukemia.

**Materials and methods**

**Sera**

Serum samples were obtained by venipuncture after informed consent from seventeen patients (8 males and 9 females; age 43 to 82 years) with CML in chronic phase treated either with hydroxyurea (HU) or interferon-α (IFN-α). Clinical characteristics of these patients at the time of the study are summarized in Table 1. Sera from 15 healthy volunteers (7 males and 8 females; age 28 to 67 years) were collected and served as normal controls. Sera were separated by centrifugation and stored at −70°C until the CSF assays were performed.

**CSF assays**

GM-CSF and G-CSF serum concentrations were determined by two specific enzyme immunoassays (EIA). GM-CSF concentrations were measured by a solid phase EIA (GM-CSF-EASIA; Medgenix Diagnostics, Fleurus, Belgium) based on the use of an oligoclonal system in which several monoclonal antibodies directed against distinct epitopes of GM-CSF are utilized, as was previously described. Briefly, a microtiter plate precoated with several anti-GM-CSF monoclonal antibodies is incubated for 4 hours at room temperature with undiluted duplicates of standards or serum samples in the presence of a different series of monoclonal anti-GM-CSF antibodies labelled with horseradish peroxidase. A chromogen (tetramethylbenzidine) is then added to detect enzymatic activity. Concentrations of GM-CSF as low as 3 pg/mL are detectable with this assay, and cross-reactivity against other cytokines such as GM-CSF, M-CSF, IL-1, IL-6, TNF-α and TNF-β.

In order to minimize variations all samples were run in the same assay, and the intra-assay coefficients of variation were 7% and 5% for GM-CSF and G-CSF, respectively.

**Statistical analysis**

The two-tailed Student’s t-test for unpaired data was used for statistical analysis of results; p values < 0.05 were considered as significant. Correlations between both G-CSF and GM-CSF concentrations and blood counts were analyzed by Spearman’s rank test.

Table 1. Patient characteristics at the time of the study.

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*IFN-α = interferon-α; HU = hydroxyurea
Results

CSF concentrations

Serum concentrations of GM-CSF and G-CSF in CML patients and normal controls are shown in Figure 1. Eight out of the 17 CML patients (44%) studied showed detectable (> 3 pg/mL) serum concentrations of GM-CSF (mean±SD 16.8±16.4; range 3.9-55 pg/mL), while only one of the healthy controls demonstrated a detectable GM-CSF level. Detectable (> 50 pg/mL) serum concentrations of G-CSF were also observed in 9 out of the 17 (52%) CML patients (mean±SD 1,063±1,240; range 150-3,540 pg/mL), while none of the normal controls showed detectable amounts of G-CSF. Interestingly enough, all 8 CML patients with detectable GM-CSF levels also presented measurable concentrations of G-CSF.

When the CML patients were evaluated according to their treatment regimen, it appeared that among the 8 patients with measurable concentrations of both GM-CSF and G-CSF, 7 were treated with HU and only one was under therapy with IFN-α; conversely, only 2 patients treated with HU and 7 patients treated with IFN-α showed undetectable levels of CSF.

Relationship between CSF and blood counts

No statistically significant correlation was observed between serum levels of either GM-CSF or G-CSF and peripheral blood counts, as shown in Table 2.

However, the patients with the highest leucocyte counts showed the highest CSF concentrations, with a trend which was very similar for both GM-CSF and G-CSF. An identical trend for a positive correlation was observed between both GM-CSF and G-CSF concentrations and absolute neutrophil counts (Figure 2).

Discussion

Our data indicate that measurable concentrations of both GM-CSF and G-CSF are detectable in the serum of a substantial number of patients with CML in chronic phase. The availability of sensitive and reliable immunoassays for both GM-CSF and G-CSF in recent years has raised interest in examining the serum levels of these cytokines in different pathological conditions. Generally, both GM-CSF and G-CSF are only rarely detected, at very low concentrations, in the serum of normal subjects. Serum levels of G-CSF have been shown to be increased during infection and have been inversely related to white cell counts in aplastic anemia and in cyclic neutropenia.

Furthermore, elevated concentrations of G-CSF have been reported in some cases of acute leukemia or after autologous bone marrow...
transplantation. A small number of studies have also investigated GM-CSF serum levels in patients affected by acute leukemias or lymphomas, without reaching any definite conclusions.

Our results are in agreement with a study by Watari et al., who found detectable amounts of G-CSF in 6 of 18 (33%) CML patients investigated. Quite interestingly, high levels of granulocyte colony-growth stimulating activity (CSA) (which was subsequently recognized as mainly constituted by both natural GM-CSF and G-CSF) were observed more than twenty years ago in the urine of patients with CML, although these results were not confirmed by others. More recently, studies at the molecular level have suggested that G-CSF mRNA is constitutively expressed by neoplastic cells in a considerable number of patients with CML either in stable or blastic phase, and that GM-CSF mRNA may be expressed by adherent bone marrow layers from CML patients in blastic phase, although other research has not confirmed these observations.

Taken together, these findings seem to suggest that endogenously secreted myeloid CSF might play a role in the pathophysiology of CML. Indeed it has been claimed that G-CSF may represent a potential autocrine growth factor in stable phase CML, and GM-CSF has been indicated as playing a central role in the pathogenesis of juvenile CML.

Besides possibly being produced by neoplastic cells as autocrine growth factors, both GM-CSF and G-CSF are certainly released by bone marrow stromal cells (fibroblasts, endothelial cells and macrophages). Marrow stroma constitutes the milieu in which the neoplastic CML clone expands, and an abnormal expression of growth factors by adherent bone marrow layers derived from CML patients has been reported.

Recent experimental evidence seems to assign a key role to over-expression of CSF in the pathogenesis of different myeloproliferative syndromes. This hypothesis has been confirmed at least in part by the observation that IFN-α is able to induce a regression of myeloid metaplasia by reducing the circulating concen-

Figure 2. Correlations between serum levels of myeloid CSF and peripheral blood leukocyte and neutrophil counts.
Serum levels of myeloid growth factors in chronic myeloid leukemia

tration of CSF-1. Moreover, IFN-γ has been shown to specifically inhibit GM-CSF expression by murine vascular endothelial cells, further confirming the direct role of IFNs in the regulation of cytokine production by accessory cells. A possible modulatory effect of IFN-α on the production or secretion of CSF by stromal or neoplastic CML cells is one of the suggested mechanisms for its antiproliferative activity in this disease, in addition to its direct inhibitory action on the growth of CML hematopoietic progenitors.

Our observation of very low serum levels of both GM-CSF and G-CSF in these patients being treated at the time of the study with IFN-α as compared to the corresponding values in those receiving HU might be consistent with such IFN-α activity. Interestingly, a sharp reduction in G-CSF serum levels has already been reported by Watari et al. in two CML patients undergoing treatment with IFN-α. Whether or not endogenous production of myeloid CSF such as GM-CSF and G-CSF is increased in untreated CML patients, and to what extent treatment affects serum concentrations of these CSF are still unknown.

In a single patient recently studied at the time of diagnosis we observed detectable serum concentrations of both GM-CSF and G-CSF (24 pg/mL and 650 pg/mL, respectively), but definition of this point clearly deserves further investigation.

As far as the relationship between myeloid CSF serum levels and the clinical characteristics of our patients is concerned, a positive trend was seen in our study toward a direct correlation between serum levels of these CSF and both white blood cell count and the number of circulating neutrophils, even though no statistically significant link emerged between either GM-CSF or G-CSF and the parameters considered. In fact, an analogous trend was described between peripheral white cell counts and urinary CSA in a study by Robinson and Pike, although here again, as well as in other such studies, no definite correlations were found.

Similarly, Watari et al. claimed that CML patients with high G-CSF levels presented clinical features like those of patients with low G-CSF concentrations. It is conceivable that the small number of patients investigated in our study and/or differences in their clinical status might account for the failure to establish a statistically significant correlation.

In short, the present research indicates that an increased secretion of myeloid CSF occurs in a substantial percentage of CML patients in chronic phase. Obviously, no definite conclusions can be drawn from this study regarding the relationship between these findings and the nature of the leukemic process. In particular, the true physiopathological implications these observations have for the mechanism of leukemic cells growth and differentiation in CML remain to be elucidated.

References