Increased Circulating CSF-1 (M-CSF) in Myeloproliferative Disease: Association With Myeloid Metaplasia and Peripheral Bone Marrow Extension

By Harriet S. Gilbert, Vincent Praloran, and E. Richard Stanley

Myeloproliferative disease (MPD) is heterogeneous in phenotypic expression and may display features consistent with expansion and activation of the monocyte/macrophage population during its course. The role of colony-stimulating factor-1 (CSF-1) in the pathophysiology of MPD was investigated by measuring circulating CSF-1 levels and examining their relationship to disease phenotype. Serum CSF-1 concentrations, measured by radioimmunoassay, were elevated in all MPD phenotypes. CSF-1 levels differed significantly between groups of patients with essential thrombocythemia, polycythemia vera, and postpolycythemic or agnogenic myeloid metaplasia (in ascending order). CSF-1 serum levels were positively correlated with spleen size and the degree of peripheral bone marrow extension, determined by scintigraphy using a macrophage-seeking isotope. There was no correlation between CSF-1 concentration and circulating levels of erythrocytes, neutrophils or platelets, or the presence of bone marrow fibrosis. Elevated serum CSF-1 levels appear to be associated with an expanded monocyte/macrophage population in MPD. In view of the known cooperativity between CSF-1 and other growth factors in regulating hematopoiesis, the finding of increased serum CSF-1 concentrations and its association with myeloid metaplasia and bone marrow extension may indicate a pathophysiologic role for CSF-1 in determining the phenotypic expression of MPD.

Chronic myeloproliferative disorders (MPD) are characterized by proliferation of a clone of pluripotent hemopoietic precursor cells (PHPCs) with a biologic advantage that permits its expansion until normal polyclonal bone marrow growth is inhibited and all functioning bone marrow is monoclonally derived.1,2 Pluripotentiality, commitment, differentiation, and maturation of the PHPC to functioning multilineage progeny are preserved. There is a reversion to a fetal distribution of hemopoietic activity, in that the "hematopoietic organ" is expanded within the central marrow cavity and there is extension of hematopoietic tissue to the marrow cavity of the long bones and extramedullary sites, such as spleen, and liver (myeloid metaplasia). The bone marrow displays varying degrees of hyperplasia, dysplasia, and fibrosis. MPD is heterogeneous in its phenotypic expression from patient to patient and in the same patient over the course of the disease. The family of syndromes that comprise chronic MPD have been classified according to disease phenotype as polycythemia vera (PV), essential thrombocytemia (ET), myeloid metaplasia (MyM), and myelofibrosis (MF).3 The factors that determine the differentiation pathways followed by the PHPC to produce these phenotypes are poorly understood. Insofar as these factors are major determinants of the patient’s clinical course and complications, their investigation can provide important insights into the biology and pathophysiology of MPD.

Previous investigations suggest the presence of an expanded and activated monocyte/macrophage population in MPD. Hypcholesterolemia is prevalent and cholesterol levels are inversely related to the degree of splenomegaly.4,5 Lipoprotein turnover studies showed an increased fractional catabolic rate of low-density lipoprotein (LDL) with a disproportionate degree of catabolism by pathways other than those mediated by receptors for native LDL.6,7 Such "nonclassical" pathways are active in macrophages. Results of imaging studies using 99mTc-technetium-labeled LDL (99mTc-LDL)8,9 support the putative role of macrophages in the accelerated LDL catabolism of MPD, since in normal subjects this radiotracer was rapidly cleared from the circulation by the kidneys, but in MPD there was localization in spleen, liver, and bone marrow in a distribution identical to that obtained with the macrophage-targeted 99mTc-sulfur colloid(99mTc-SC). Marrow that was replaced by fibrous tissue showed no uptake of LDL or sulfur colloid, indicating that the bone marrow fibroblast population was not directly responsible for increased LDL catabolism. The demonstration of similar abnormalities of cholesterol metabolism in Gaucher disease, a disorder characterized by an expanded and activated macrophage population in the absence of a malignant disorder of hematopoiesis, lent further support to the postulated role of an expanded and stimulated macrophage population in producing hypcholesterolemia.10 Increased serum lysozyme concentrations in patients with MPD11 provide further evidence of an expanded macrophage population in this disorder.

Macrophage production is mediated by colony-stimulating factor-1 (CSF-1), a hematopoietic growth factor (HGF) that stimulates the survival, proliferation, and differentiation of mononuclear phagocytes (precursor cell → monocyte → promonocyte → monocyte → macrophage), which selectively express high levels of the CSF-1 receptor. CSF-1 regulates the survival of nondividing macrophages and the survival, proliferation, and differentiation of the precursor cells.12,13 It also synergizes with other HGFs to stimulate the proliferation and differentiation of developmentally earlier stages of mononuclear phagocytes.

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cells that express low levels of the CSF-1 receptor.\(^\text{14,15}\) An increase in circulating CSF-1 concentration might be expected to stimulate monocyte/macrophage differentiation, proliferation, and survival, thereby expanding and increasing the function of this population in MPD. Since CSF-1 is present in serum in concentrations that are detectable by radioimmunoassay,\(^\text{16}\) this hypothesis was tested by measuring circulating CSF-1 levels in MPD and determining their relation to disease phenotype.

**MATERIALS AND METHODS**

*Patient material.* Serum was obtained from blood samples drawn with informed consent from age-matched, hematologically normal subjects and patients with MPD who met the diagnostic criteria of the Polycythemia Vera Study Group.\(^\text{3}\) The protocol was approved by the local Committee for Clinical Investigation. Disease activity was evaluated by a comprehensive medical history, physical examination, complete blood count, serum chemistry determinations, bone marrow biopsy, serum unsaturated vitamin B\(_2\) binding capacity, and liver, spleen, and bone marrow imaging with \(^{99m}\text{Tc-SC.}\)

Patients were categorized by MPD phenotype as essential thrombocythemia (ET), polycythemia vera (PV), postpolycythemic myeloid metaplasia, and agnogenic myeloid metaplasia (AMyM). Subjects were free from clinically evident infection and were not receiving myelosuppressive treatment at the time of study, except for two patients with PV and one with AMyM, who were being treated with hydroxyurea. Bone marrow biopsies were examined for the presence of collagen fibrosis according to published criteria.\(^\text{17}\)

*CSF-1 assay.* Serum CSF-1 was measured using a modification of a previously published radioimmunoassay (RIA).\(^\text{18}\) Modifications improved the sensitivity of the assay and included slight changes in the pH and protein concentration of the buffers and a change in the technique used for precipitation of the CSF-1 antigen-antibody complexes. Purified human CSF-1 from medium conditioned by the M1A PaCa cell line or recombinant CSF-1 was obtained from Cetus Corp (Emeryville, CA) and iodinated as described.\(^\text{19}\) Anti-human CSF-1 antiserum was raised in rabbits by immunization with purified human urinary CSF-1.\(^\text{20}\) Normal rabbit serum (NRS) diluted 1/10 in RIA buffer, \(pH 7.5,\) was used for the preparation of the 1/1,000 dilution of antibody. Protein concentrations of standards and samples were kept equivalent by diluting CSF-1 standards and samples (with low protein concentrations) in NRS 1/10 and serum samples in RIA buffer at 1/10. Higher dilutions of serum were made in NRS 1/10. Separation of CSF-1 antigen-antibody complexes from free CSF-1 was effected by precipitation with 16\% polyethylene glycol (PEG) in 50 mmol/L sodium phosphate buffer, \(pH 7.5,\) at room temperature. A two-step assay\(^\text{18}\) was performed in which the antiserum and test samples were incubated for 20 hours at room temperature, followed by addition of \(^{125}\text{I-CSF-1}\) and incubation for a further 20 hours at room temperature prior to addition of PEG. These modifications decreased the nonspecific precipitation of \(^{125}\text{I-CSF-1}\) to <10\% of the total \(^{125}\text{I-CSF-counts.}\) The assay reproducibly measured CSF-1 in the range 0.42 to 27 fmol/tube. The concentration range for measurement of CSF-1 in serum was 30 pg/ml (1 pmol/L) to 12 ng/ml (400 pmol/L). The improvement in reproducibility of immunoassay results conferred by diluting serum tenfold to avoid interferences from contaminating proteins has been confirmed by Hanamura et al for measurement of human monocyte colony-stimulating factor by enzyme-linked immunosorbent assay.\(^\text{19}\) The assays reported in the present study were performed using biologic CSF-1. More recent data using rhCSF-1 gave comparable results.

*Statistics.* Populations showing a normal distribution of CSF-1 concentrations were compared by Student’s \(t\)-test. Populations with values not normally distributed were analyzed by Wilcoxon rank sum testing. Differences were considered significant at \(P < .05.\)

**RESULTS AND DISCUSSION**

Serum CSF-1 concentrations were measured in duplicate in 34 hematologically normal subjects and 49 patients with MPD. Serum CSF-1 levels, grouped by MPD phenotype, are shown in Fig 1 and Table 1. Values were normally distributed in all but the AMyM group. All MPD phenotypes showed significant elevations of serum CSF-1 when compared with the age-matched, hematologically normal population. Within the MPD population, there were significant differences in serum CSF-1 concentrations amongst the ET, PV, and the combined PPMyM and AMyM groups, ranked in ascending order. There was no correlation between serum CSF-1 concentration and circulating levels of erythrocytes, platelets, total leukocytes, or neutrophils in the MPD population (data not shown). Higher levels of CSF-1 were observed in MPD phenotypes with myeloid metaplasia. Palpable spleen size, measured as centimeters of vertical expanse at the left midclavicular line, was significantly greater in PPMyM and AMyM (mean 16, SD 7.2, median 17 cm) v ET and PV (mean 2.6, SD 2.5, median 2 cm, \(P < .001\)) and serum CSF-1 concentrations and spleen size were positively correlated (\(r = .537, P < .001\)). Elevated CSF-1 concentrations were not solely dependent on the physical presence of the spleen, since two patients who had undergone splenectomy for myeloid metaplasia also had increased levels (10.7, 18.2 ng/mL). Both patients had other evidence of active myeloproliferative disease with myeloid metaplasia (hepatomegaly and peripheral bone marrow expansion).

Peripheral bone marrow expansion was determined in 43 patients with MPD by scintigraphy using the macrophage-seeking isotope, \(^{99m}\text{Tc-SC.}\) Expansion was categorized in the

![Fig 1](image-url)
lower extremities as grade 1 (normal; no scintigraphic activity below the upper third of the femur); grade 2 (expansion to femur; scintigraphic activity extending to the lower femur and knee; grade 3 (expansion to femur and tibia; scintigraphic activity extending to the lower third of the tibia or below). MPD patients with bone marrow expansion involving the entire lower extremity had significantly higher bevels of serum CSF-1 than those without peripheral marrow expansion and those with marrow expansion limited to the femur (Fig 2). Since the upper third of the femur normally contains some hematopoietic tissue, when considered in terms of bone marrow and those with marrow expansion limited to the femur and those with expansion involving only the femur would represent a significantly greater increment in hematopoietic tissue than would expansion only to the middle and lower third of the femur. If bone marrow expansion and CSF-1 concentrations are related, the observed significant increase in CSF-1 levels in patients with expansion involving the entire lower extremities would be expected.

Since fibroblasts are a source of CSF-1 and reactive fibroblastic proliferation is a complication of MPD often associated with myeloid metaplasia, the presence of collagen fibrosis was determined from bone marrow biopsies of the posterior iliac crest and the relation of serum CSF-1 concentrations to the presence of fibrosis in the 17 patients with myeloid metaplasia was examined. There was no significant difference in CSF-1 concentrations between patients in the myeloid metaplasia group with (n = 9) and without (n = 8) bone marrow fibrosis (mean ± SD, 11.4 ± 4.3 vs 12.9 ± 7 ng/mL, respectively; P > .05).

The lack of correlation between serum CSF-1 concentrations and leukocyte counts in the MPD population is notable and is in agreement with our findings in four patients with leukemia (two acute myeloblastic, one acute lymphoblastic, one chronic myelocytic) before and during intensive chemotherapy. All had normal CSF-1 levels before therapy and showed no significant change during treatment (Praloran V, Gilbert HS, Stanley ER, unpublished observations). Hanamura et al. reported elevated serum M-CSF levels in one half of a group of patients with hematologic malignancy after anticancer chemotherapy, and postulated that this was a response to neutropenia. The persistence of normal M-CSF levels in the other half was attributed to significant damage to CSF-producing cells by intensive chemotherapy. Serum M-CSF was also elevated in patients with infection and varying degrees of neutrophilia, as well as in pregnant women with normal neutrophil counts.

Elevated serum CSF-1 could result from increased production and/or decreased catabolism. The effects and turnover of circulating CSF-1 are mediated by the CSF-1 receptor present on the target populations. This receptor is encoded by the c-fms protooncogene and expressed selectively in normal mononuclear phagocytes. The cellular source of circulating CSF-1 in humans is poorly understood. While monocytes express low levels of CSF-1 transcripts, monocyte activation by phorbol ester induces CSF-1 expression. This suggests that under certain conditions monocytes may have the capacity to autostimulate certain functions through the production of CSF-1. Treatment of resting human monocytes with GM-CSF similarly induces CSF-1 expression. Induction of CSF-1 transcripts in monocytes is associated with synthesis of the CSF-1 gene product. This induction occurred during the continued expression of c-fms transcripts, although there was a partial down-regulation of c-fms transcription. Other cytokines, including T-cell–derived γ-interferon and interleukin-3, appear capable of inducing CSF-1 in monocytes. Based on the behavior of normal human monocytes, a hypothesis to explain increased circulating concentrations of CSF-1 in MPD would be the presence of an expanded and activated monocyte/macrophage population in which CSF-1 production is increased, either by an autocrine mechanism or by induction by other HGFs, and CSF-1 receptor (c-fms) expression is down-regulated, leading to decreased removal and catabolism of circulating CSF-1. The alterations in the metabolism of CSF-1 and other hematopoietic growth factors and cytokines

### Table 1. Serum CSF-1 Concentrations (ng/mL) in Hematologically Normal Subjects and Patients With Myeloproliferative Syndromes

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>34</td>
<td>4.9 ± 1.1</td>
<td>4.8</td>
<td>1.7–7.1†</td>
<td>.04</td>
</tr>
<tr>
<td>ET</td>
<td>9</td>
<td>5.9 ± 1.3</td>
<td>6.0</td>
<td>3.4–6.1†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PV</td>
<td>23</td>
<td>7.4 ± 1.8</td>
<td>7.4</td>
<td>3.6–10.7†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PPMym</td>
<td>7</td>
<td>12.4 ± 3.3</td>
<td>10.8</td>
<td>9.0–18.2†</td>
<td>.001</td>
</tr>
<tr>
<td>AMym</td>
<td>10</td>
<td>12.0 ± 7.2</td>
<td>10.2</td>
<td>6.5–31.6‡</td>
<td>.005</td>
</tr>
</tbody>
</table>

*Compared with Normal group.
†Population normally distributed.
‡Population not normally distributed.

Fig 2. Relation of CSF-1 levels to peripheral bone marrow expansion in MPD. Bone marrow expansion was determined by scintigraphic activity in the lower extremities after injection of technetium sulfur colloid. Serum CSF-1 concentrations of 19 patients with no peripheral extension of bone marrow (NONE), 12 with extension involving the femur, and 12 with extension involving both femur and tibia are represented by scatter plots and box plots. The center line of the box is the median that splits the population in half. The edges of the box are the hinges that split the halves in half again and demarcate the 25th to 75th percentile of the population. Outliers with values falling beyond 1.5 time the range between the hinges are indicated by an asterisk. The vertical lines extend from the hinges to the largest or smallest nonoutlier value. Intergroup comparisons by Wilcoxon signed ranks testing showed significantly higher CSF-1 values in patients with peripheral bone marrow expansion to the femur and tibia compared with those with no expansion (P < .002) and with expansion to the femur (P < .004). CSF-1 concentrations were not significantly different in the group without peripheral expansion and those with expansion involving only the femur (P = .06).
that might alter CSF-1 metabolism in MPD remain to be investigated.

The elevated serum CSF-1 in MPD could result from either increased release into or decreased clearance from the circulation, or both. The cellular and tissue sources of circulating CSF-1 are not known and may vary in different imposed situations. However, in mice, clearance of circulating CSF-1 occurs by CSF-1 receptor-mediated endocytosis and intracellular destruction by macrophages of the liver and spleen. Elevated plasma CSF-1 concentrations observed in MPD could, therefore, result from a decrease in the number and/or function of these macrophages. Whatever the cause of increased CSF-1 levels in MPD, their association with myeloid metaplasia and bone marrow expansion suggests that CSF-1 may affect the amount and distribution of hematopoietically active tissue in MPD by direct stimulation of the macrophage population and/or by stimulation of the PHPC and its other progeny through cooperativity with other HGFs. The availability of sufficient quantities of recombinant human M-CSF for human administration will facilitate studies of the biology of CSF-1 in MPD to test these hypotheses and obtain insights into potential clinical applications of CSF-1 for manipulation of the hematopoietic organ.

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REFERENCES

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