Increased Macrophage Colony-Stimulating Factor Levels in Immune Thrombocytopenic Purpura


Thrombocytopenia is a dose-limiting toxicity of macrophage colony-stimulating factor (M-CSF) in preclinical and initial phase I trials. Modulation of macrophage-mediated platelet destruction in immune thrombocytopenic purpura (ITP) may be affected by M-CSF activity. In this study, plasma levels of M-CSF were determined by a sensitive radioimmunoassay in 23 patients with ITP. These were compared with control levels measured in 24 healthy subjects. M-CSF levels were significantly higher in the ITP patients than in the control subjects (218 ± 179, P < .02); however, there was a great deal of overlap. The highest M-CSF levels (median = 299 U/mL) were observed in three patients with Evan’s syndrome. Patients with severe ITP (platelets <25,000/μL) had intermediate M-CSF levels (median = 231 U/mL) and those with mild thrombocytopenia (>25,000/μL) had normal levels (median = 173 U/mL). Sixteen patients were treated with corticosteroids: 10 responded and 6 did not. Median M-CSF levels were higher in those who failed to respond compared with responders (272 ± 202, P < .05). These findings suggest M-CSF may influence macrophage-mediated platelet destruction in ITP.

Control Patient Characteristics

Two groups of control thrombocytopenic patients with platelet counts ≤25,000/μL also had their M-CSF levels measured. The first group consisted of five patients with megakaryocytic aplasia. Three of these patients had severe aplastic anemia and two had poor bone marrow engraftment after an autologous bone marrow transplant for acute myelogenous leukemia. The second control group consisted of five patients with primary thrombotic thrombocytopenic purpura. All patients had microangiopathic hemolytic anemia and thrombocytopenia associated with elevated LDH levels (range 548 to 1,184 U/L). Three of the five had fluctuating neurologic symptoms.

Materials and Methods

M-CSF assay. Plasma anticoagulated with EDTA was stored at −70°C before assay of M-CSF levels. Samples from control subjects were treated identically. Plasma M-CSF activity was measured using a sensitive and specific double-antibody radioimmunoassay,22 The present assay differs from that described previously by substituting recombinant human M-CSF (Cetus, Emeryville, CA; 4.3 × 10⁹ U/mg protein) and using an Iodo-Bead (Pierce, Rockford, IL) method for iodination of the tracer. In this technique, duplicate 0.1-mL samples of plasma are added to antibody (sheep anti-rabbit IgG) at 1:30,000 dilution in 5% albumin. Radioiodinated human M-CSF is added of rabbit anti-CSF serum. Radioiodinated human M-CSF is added to the CSF complex. The precipitate is concentrated by centrifugation and assessed by gamma counter.

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Iodination of the M-CSF is accomplished using the Iodo-Bead reagent. A single Iodo-Bead is washed with 0.5 mL of 0.2 mol/L phosphate buffer, pH 7.0, and dried on filter paper. This is added to a 3-mL reactivial (Pierce) and moistened with 20 μL of the phosphate buffer containing 0.6% polyethylene glycol molecular weight 4000 (PEG). One millicurie of Na \(^{125}\)I (DuPont, Boston, MA) (2 μL) is added and the mixture is held at 0°C for 5 minutes. Iodination is accomplished by addition of 1.4 μL of a 1:10 dilution of DM50 and 2 μg of recombinant M-CSF (Cetus) in 15 μL of buffer. After 30 minutes at 0°C, the reaction is terminated by addition of 1.3 μL of potassium metabisulfite. The iodinated M-CSF is diluted in 0.05 mol/L Tris-PEG buffer and separated from free iodine on a column of Sephadex 6-25 (Pharmacia, Piscataway, NJ); whereas, surface IgG was measured on patients not treated with Trion X (eg, without lysis).

Platelet count. Platelet counts were performed by the method of Brecher and Cronkite\(^{14}\) and were confirmed by phase-contrast microscopy.

STATISTICS

Mann-Whitney tests were used to compare medians; a \( P \) value <.05 was considered significant. The Pearson product moment correlation was used to calculate correlations.

RESULTS

The median concentration of plasma M-CSF levels in the normal controls was 179 U/mL (range 151 to 219 U/mL). There was no difference between males and females.

The median levels in ITP patients was greater than in the normals. Median M-CSF levels were 218 (range, 141 to 405 U/mL) \(( P < .01)\).

M-CSF levels did not correlate with PaIgG \(( r = .25)\) or the degree of thrombocytopenia \(( r = .23)\). The degree of thrombocytopenia also did not correlate with PaIgG \(( - .42)\).

To further understand differences in M-CSF levels, three groups of patients were identified. Patients with mild ITP (platelet count >25,000/μL) had normal M-CSF levels with a median value of 173 U/mL \(( r = 141 \) to 229). Patients with severe ITP (platelet counts <25,000/μL) had intermediate M-CSF levels with a median value of 231 U/mL and a range of 176 to 323.8 U/mL \(( P < .001)\). Patients with Evan's syndrome had the highest M-CSF levels with a median value of 299 U/mL and a range of 234 to 405 U/mL (see Fig 1).

CONTROL GROUPS OF THROMBOCYTOPENIC PATIENTS

M-CSF levels were also measured in two control groups with platelet counts ≤25,000/μL. The first group had megalakroytic anemia associated with severe aplastic anemia (three patients) and with poor engraftment postautologous bone marrow transplantation (two patients). M-CSF levels were normal in these patients with a median value of 180 U/mL \(( r = 128 \) to 191 U/mL).

The second control group consisted of five patients with primary thrombotic thrombocytopenic purpura (TTP). Unlike the patients with ITP and platelets ≤25,000/μL, M-CSF levels were also normal in the patients with TTP; their M-CSF value was 189 with a range of 152 to 212 U/mL.

The median M-CSF level in 11 patients with ITP and platelet counts ≤25,000/μL was 231 (range 176 to 324 U/mL). M-CSF levels were higher in the patients with severe ITP \(( ≤25,000/μL)\) compared to those patients with either megalakroytic aplasia \(( P < .01)\) or with TTP \(( P < .01)\).

RELATIONSHIP OF M-CSF LEVEL TO CLINICAL RESPONSE IN ITP

The relationships of circulating M-CSF levels to clinical response to corticosteroids (16 patients) or splenectomy (9 patients) were also compared (Fig 2). Of 16 patients who were initially treated with prednisone (60 to 80 mg/d), six failed to increase their platelet count by 30,000/μL from baseline. The median M-CSF level of these patients was 271 U/mL. Ten patients were more responsive with platelet increments of ≥30,000/μL after initial treatment with prednisone. Their median M-CSF level was 202 U/mL (\( P < .05)\).

Nine patients underwent splenectomy. Five achieved a sustained complete response. The median M-CSF level was 231 (218 to 324) in the complete responders. The median M-CSF level in the patients who had poor responses to splenectomy was similar at 231 (212 to 251 U/mL) \(( P = \text{not significant})\).

DISCUSSION

The binding of the Fc portion of IgG to platelets and/or red blood cells plays a major role in cellular destruction in autoimmune thrombocytopenia purpura (ITP) and hemolytic anemia (AIHA). The present studies measured levels of the cytokine, M-CSF, in a series of ITP patients. The highest levels were seen in those patients with Evan's syndrome (combined ITP and AIHA). ITP patients with mild chronic ITP had normal M-CSF levels. By contrast, patients with more severe ITP had elevated M-CSF levels. M-CSF levels
were normal in two thrombocytopenic control groups, eg, those with severe megakaryocytic aplasia and severe TTP. Therefore, the increase in M-CSF levels does not appear to be a secondary phenomenon in response to thrombocytopenia or to increased clearance of platelets.

A correlation was seen between M-CSF level and clinical response to corticosteroids. Steroid nonresponders had higher M-CSF levels than responders. These results suggest that high levels of M-CSF may contribute to or perhaps initiate platelet destruction by affecting macrophage function in some patients with ITP or Evan’s syndrome. Several recent studies have focused on monocytic function, which appears increased in ITP15,17 and AIHA.16 Monocyte Fc receptor numbers or function are increased in ITP17 and AIHA16 and monocytic-platelet rosette formation is increased in patients with refractory ITP.15

M-CSF increases ADCC in tumor cell systems19,20 and increases the expression of low-affinity IgG receptors on monocytes.19,20 Low-affinity IgG receptors may be very important in ITP, as there is a report of a refractory ITP patient who responded to therapy using a monoclonal antibody directed against a low-affinity 51 to 73/Kd Fcγ receptor.25

In the present study, high M-CSF levels were seen in patients with more severe and steroid refractory ITP. Therefore, high M-CSF levels may promote increased monocytic phagocytosis in these patients, perhaps by modulating Fc receptors. The correlation with lack of steroid response is interesting. Steroids are viewed as having several actions in ITP. For example, steroids have been shown to affect Fcγ receptor expression and to decrease the clearance of IgG-coated platelets and/or red blood cells.3,16 However, Gernsheimer et al14 have recently shown that prednisone improves platelet counts in ITP primarily by improving platelet production rather than by suppressing platelet destruction.

M-CSF has been administered to animals and to patients with malignancies. In nonhuman primates, M-CSF infusions produced increased monocyte counts, erythropagocytosis, and thrombocytopenia associated with an increase in megakaryocytes.19 In humans, thrombocytopenia has been observed as a dose-related toxicity.20,21 The highest M-CSF levels in this series were seen in those patients with Evan’s syndrome. This occurred despite platelet counts in these individuals that were only moderately decreased. ITP patients with similar degrees of thrombocytopenia had normal M-CSF levels. The primate studies suggest that M-CSF may influence both red blood cell and platelet destruction. Because red blood cells are larger than platelets, they may be preferentially removed when ITP and AIHA occur simultaneously.

Taken together, these results suggest that M-CSF levels may enhance macrophage activity and platelet and/or red blood cell destruction in ITP and/or AIHA. These observations suggest possible novel therapeutic approaches in a subset of patients with autoimmune cytopenias.

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