Amegakaryocytic thrombocytopenia (AMT) is a rare and often fatal disorder of infancy and childhood presenting with isolated thrombocytopenia that progresses to marrow failure. The defect in thrombopoiesis is not well understood nor is the etiology of the progressive marrow failure. No standard modality of treatment exists. Here, we evaluated the capacity of marrow cells isolated from five patients with AMT and progressive marrow failure to generate megakaryocyte progenitor cells (CFU-MK). These in vitro studies demonstrated assayable numbers of CFU-MK from all patient bone marrows that responded in vitro to the addition of interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), or the combination of both. These findings suggest that the defect in AMT might be partially correctable by the administration of these cytokines. A Phase I/II trial of in vivo administration of these same hematopoietins in the identical patients was conducted in which no significant toxicity was observed. IL-3 but not GM-CSF administration resulted in improved platelet counts in two patients and decreased bleeding and transfusion requirement in the remaining three. No clinical benefit was observed when GM-CSF was administered after IL-3 pretreatment. Prolonged IL-3 administration has resulted in platelet increases in an additional two patients. In vitro responsiveness of CFU-MK to either cytokine did not predict the degree of clinical response. Although the optimal dose and schedule of IL-3 either alone or in combination remains to be established, this study suggests that IL-3 may contribute to the treatment of patients with AMT.
NALDT—bone marrow subpopulations were assayed for their ability to produce megakaryocyte colony forming units (CFU-MK)–derived colonies using a serum-depleted fibrin clot assay system. 10–14 As mentioned above, various cytokines were used alone or in combination as sources of megakaryocyte colony-stimulating activity (MK-CSA). Cultures were incubated for 12 days at 37°C in a 100% humidified atmosphere of 5% CO2 in air. After incubation, fibrin clots were fixed in situ in methanokacetone (0.32 mol/L sucrose, 10 mmol/L Tris-HCl pH 7.5, 0.5 mmol/L MgCl2, 1% triton X-100). The nuclei were digested with SDS/proteinase K. DNA was extracted with phenol/chloroform as per Bell et al. 15 The DNA was then precipitated in ethanol twice and resuspended in H2O or TE before amplification. Screening for PGK heterozygotes and subsequent clonality analysis were performed using the polymerase chain reaction as described by Gilliland et al. 16

Recombinant human IL-3 and GM-CSF. The IL-3 and GM-CSF administered in this study were provided by Hoechst-Roussel Pharmaceutical Inc (Somerville, NJ). The IL-3 cDNA clone was expressed in yeast cells (Saccharomyces cerevisiae) as a single chain, glycosylated polypeptide with a relative molecular weight of 17,000 ± 5,000. The GM-CSF cDNA was also expressed in yeast cells as a single-chain polypeptide with molecular masses of 19,500, 16,800, and 15,500 Kd. Molecular weight differences are apparently due to variations in the degree of glycosylation.

Patient characteristics. Patients were eligible for study if they were thrombocytopenic without other known etiology or predisposing cause, had radiologically normal radii, were found to be thrombocytopenic without other known etiology or predisposing cause, and had an adequate response to transfusion. Each time 2 to 10 mL of blood were obtained and processed. Granulocytes were separated from mononuclear cells by density gradient centrifugation on Hypaque-Ficoll. Granulocyte nuclei were then isolated by lysing cells with triton X-100 solution (0.32 mol/L sucrose, 10 mmol/L Tris-HCl pH 7.5, 0.5 mmol/L MgCl2, 1% triton X-100). The nuclei were digested with SDS/proteinase K. DNA was extracted with phenol/chloroform as per Bell et al. 15 The DNA was then precipitated in ethanol twice and resuspended in H2O or TE before amplification. Screening for PGK heterozygotes and subsequent clonality analysis were performed using the polymerase chain reaction as described by Gilliland et al.16

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Patient characteristics. Patients were eligible for study if they were thrombocytopenic without other known etiology or predisposing cause, had radiologically normal radii, were found to be thrombocytopenic in the first year of life and had absent to severely reduced megakaryocytes on bone marrow biopsy. Patients were also required to have normal or near normal renal and hepatic function, no evidence of anti-platelet antibodies, and be ineligible for or refuse bone marrow transplantation. Five children were enrolled (Table 2); three were platelet transfusion dependent and two were also red cell (RBC) transfusion dependent at study entry. Four of five patients had an absolute neutrophil count < 1.5 × 109/L. All had decreased hematocrit for age and four of five had macrocytic RBCs. The study was approved by the Committee for the Protection of Human Subjects, The Children’s Hospital (Boston, MA). Informed consent was obtained from all patients and/or their parents or guardians.

Clinical study design. IL-3 was administered at 62.5 µg/m2 by twice subcutaneous (SC) bolus daily for 21 days. Patients failing to have an adequate response (defined below) after 21 days had their dose doubled to 125 µg/m2 twice daily SC. Patients still failing to have an adequate response received a third 21-day cycle consisting of 7 days of IL-3 at 62.5 µg/m2 twice daily SC followed by 14 days of GM-CSF at 250 µg/m2 every day SC. Patients with hematologic...
EFFECTS OF IL-3 AND GM-CSF ON AMT

Improvement were maintained on individualized schedules of IL-3 and/or IL-3 and GM-CSF after cessation of the study.

For purposes of dose or cycle escalation only, response was defined as a doubling of the platelet count if the initial count was \( >25 \times 10^9 \) L or the achievement of a platelet count \( >50 \times 10^9 \) L, or the achievement of a platelet count \( >25 \times 10^9 \) L. The neutrophil count was defined as the sum of peripheral neutrophils, bands, metamyelocytes, and myelocytes.

**Laboratory evaluation.** Before and during drug administration, patients were evaluated by complete history and physical and laboratory examinations, which consisted of a chest radiograph, electrocardiogram, complete blood cell count with differential, reticulocyte count, serologic biochemical profile, iron studies, peripheral blood immunophenotyping, antiplatelet antibodies, and urinalysis. Bone marrow aspirates and biopsies were also obtained at predetermined intervals.

**RESULTS**

Marrow specimens from all five patients were examined for clonality and for in vitro response of megakaryocyte progenitors to IL-3 and GM-CSF before in vivo treatment with these cytokines. Subsequently, all five patients completed each of the required three treatment cycles and were evaluable for in vivo response and toxicity.

**IL-3 and GM-CSF induce megakaryocyte progenitor colony formation in vitro.** The effect of IL-3 and GM-CSF either alone or in combination, on the formation of CFU-MK by nonadherent, low density, T-depleted marrow cells isolated from normal donors is shown in Fig 1. Small numbers (3.8 ± 0.6) of CFU-MK-derived colonies formed in the absence of any exogenous cytokine. The addition of either GM-CSF (200 pg/mL) or IL-3 (1 ng/mL) to the culture promoted a four to five-fold increase in colony formation \( (P < .05) \). The combination of IL-3 and GM-CSF led to the appearance of approximately two to three times greater numbers of CFU-MK-derived colonies than was observed with either cytokine alone \( (P < .05) \). The capacity of nonadherent, low density, T-depleted marrow cells isolated from five patients (A through E) with AMT to form CFU-MK-derived colonies is also depicted in Fig 1. Numbers of CFU-MK assayed from the marrows of patients A through D, in the absence of exogenous cytokines, was approximately 50% of that observed in cultures from normal marrow \( (P < .05) \). Similarly, four to five-fold greater numbers of CFU-MK-derived colonies formed in response to the addition of either IL-3 or GM-CSF in patients A through D when compared with unstimulated cultures from the same patients. This change was significantly less dramatic than was seen in normal donors \( (P < .05) \). In patients A and B, but not in C or D, the stimulatory activity of IL-3 and GM-CSF on CFU-MK colony formation was additive, albeit only to 50% to 80% of the level observed in normal marrow.

In contrast to the response of the above four patients, marrow cells isolated from patient E were significantly more responsive to both IL-3 and GM-CSF. Baseline CFU-MK-derived colony formation was approximately sixfold greater than that observed with normal marrow cells and approximately 12-fold greater than observed in patients A through D. Additionally, marrow cells from this patient formed three times as many CFU-MK colonies in response to IL-3, five times as many in response to GM-CSF and almost three times as many in response to the combination of IL-3 and GM-CSF as did marrow from normals (Fig 1). This patient’s marrow responses to IL-3 and/or GM-CSF ranged from 3- to 12-fold greater than that observed in the other four patients.

Examination of CFU-GM colony formation revealed significantly fewer colonies in cultures of patient marrow (range 0.0 to 1.0 ± 0.0) than in cultures of normal marrow (15.5 ± 0.9). The addition of either GM-CSF or IL-3 alone resulted in increased CFU-GM colony formation in both patients and controls. In cultures of normal marrow, the combination of GM-CSF and IL-3 resulted in a two-fold increase in CFU-GM colony formation when compared with either agent alone. However, this combination was no more effective than either agent alone in cultures of patient marrow (data not shown). The total number of colonies formed by patient marrow exposed to both GM-CSF and IL-3 (range 7.0 to 12.0 ± 1.4) remained significantly less than that generated

**Fig 1.** Effect of no addition, and IL-3 or GM-CSF alone or in combination on CFU-MK-derived colony formation by 1 × 10⁶ NALDT–bone marrow cells obtained from either normal controls or from five patients (A through E) with AMT. Each point represents the mean ± the SEM of data obtained from one to five separate experiments performed in duplicate.
by cultures of normal marrow treated in the same way (40.8 ± 0.6).

Granulocytes from patients with AMT are not clonal before or after cytokine therapy. AMT is one of the marrow failure syndromes that carries an increased risk for development of leukemia. Although all five patients had normal karyotypes by conventional cytogenetics, we wished to look for early evidence of clonal expansion. In addition, the potential of hematopoietins to expand or induce leukemic proliferation before and throughout the treatment course. Three of the five patients were found to be informative at the POK locus. All three patients had polyclonal granulocytes before treatment and remained polyclonal on subsequent analysis either during treatment or after completion of treatment.

IL-3, but not GM-CSF, induces thrombopoiesis in AMT patients in vivo. All patients exhibited varying degrees of improved thrombopoiesis in vivo following IL-3 administration, but not during GM-CSF, administration (Table 3). For purposes of illustrating differences between treatment with IL-3 alone and treatment with IL-3 followed by GM-CSF, the hematologic results will be reported: (1) for the 6-week period of escalating IL-3 (125 µg/m²/d for 3 weeks, cycle 1, followed by 250 µg/m²/d for 3 weeks, cycle 2) and (2) for the 3-week cycle of sequential treatment (IL-3 125 µg/m²/d for 7 days followed by GM-CSF 250 µg/m²/d for 14 days, cycle 3).

During treatment with IL-3 alone (cycles 1 and 2), two patients (cases B and C) had a significant increase in peripheral platelet count to 37 and 42 × 10⁹/L, respectively, from baselines of 10 and 17 × 10⁹/L. These responses are illustrated in Fig 2, which contrasts the patients pretreatment and cytokine-stimulated platelet counts. Peak platelet counts were attained during cycle 1 (Fig 2). The mean platelet count was maintained at an elevated level through cycle 2 (Table 3), although no further increase was observed despite dose escalation of IL-3 (Fig 2, Table 3). These two patients continued to have minimal evidence of active bleeding. In contrast, patients A and D, both of whom had histories of hemorrhage (Table 2) as well as evidence of active bleeding, experienced decreases in bruising, petechiae, gingival bleeding, and epistaxis (Table 3). These changes were seen in the context of decreased transfusion requirements although no increase in peripheral platelet counts could be demonstrated. Clinical changes in patient E were difficult to evaluate because of her frequent transfusion schedule and high, transfused platelet count.

In response to IL-3, the median white blood count (WBC) increased from 4.8 × 10⁹/L (range 4.2 to 6.6) at study entry to a peak value of 8.0 × 10⁹/L (range 5.8 to 10.4) during cycle 1 and a peak value of 9.2 × 10⁹/L (range 5.9 to 10.6) during cycle 2 (Fig 3A). The median absolute neutrophil count (ANC) rose from 1.15 × 10⁹/L (range .42 to 2.9) at study entry to peak values of 3.4 × 10⁹/L (range 2.1 to 5.1) during cycle 1 and 3.3 × 10⁹/L (range 1.7 to 9.1) during cycle 2 (Fig 3B). The eosinophil count of each patient increased, but this was highly variable and did not necessarily increase with time. Median absolute eosinophil counts rose from a median of 0 × 10⁹/L at baseline to maximal values of .27 ×

---

**Table 3. Responses to Treatment: Platelet Count and Platelet Transfusion Requirement**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Status in Month Before Study</th>
<th>Type of Bleeding</th>
<th>Status During Treatment</th>
<th>Status in Month Before Study</th>
<th>Type of Bleeding</th>
<th>Status During Treatment</th>
<th>Mean PLT Count/Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency</td>
<td>No. of PLT TX/Cycle</td>
<td>Bleeding Frequency</td>
<td>No./PLT TX/Cycle</td>
<td>Bleeding Frequency</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Epistaxis</td>
<td>2-5 times daily;</td>
<td>NA</td>
<td>After 1 wk, no mucosal</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Gingival Conjunctival</td>
<td>massive</td>
<td></td>
<td>bleeding, skin much</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Petechiae Purpura</td>
<td>spontaneous</td>
<td></td>
<td>better</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>Petechiae</td>
<td>Occasional</td>
<td>0</td>
<td>Occasional</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>Petechiae Purpura</td>
<td>Both always</td>
<td>0</td>
<td>Occasional petechiae,</td>
<td>0</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>present on</td>
<td></td>
<td>single bruises</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>extremities and</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>D</td>
<td>Epistaxis</td>
<td>2-4 times/wk;</td>
<td>4</td>
<td>1-2 times/mo; bruises</td>
<td>1</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Mucous membrane</td>
<td>always</td>
<td></td>
<td>with trauma only,</td>
<td>0</td>
<td>1†</td>
<td>12†</td>
</tr>
<tr>
<td></td>
<td>Petechiae Purpura</td>
<td>spontaneous</td>
<td></td>
<td>occasional petechiae</td>
<td>0</td>
<td>1†</td>
<td>4†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>skin bleeding</td>
<td></td>
<td></td>
<td>0</td>
<td>1†</td>
<td>16†</td>
</tr>
<tr>
<td>E</td>
<td>Petechiae</td>
<td>Occasional</td>
<td>4†</td>
<td>Occasional petechiae,</td>
<td>3‡</td>
<td>1</td>
<td>6‡</td>
</tr>
<tr>
<td></td>
<td>bruises with trauma</td>
<td></td>
<td></td>
<td>occasional bruises</td>
<td>1</td>
<td>1†</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td>Purpura</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>1†</td>
<td>NE</td>
</tr>
</tbody>
</table>

Patient A transfused rarely despite severe bleeding because of lack of platelets in native country and platelet refractoriness.

Abbreviations: NA, not applicable; NE, not evaluable because of transfusion schedule as noted above.

* Mean PLT count, average of all counts obtained within the 21 day cycle.

† Transfused electively to avoid potential bleeding at mother's wedding.

‡ Last plt count pre-transfusion.

§ Patient E transfused electively at relatively higher counts because of history of CNS hemorrhage and hemiparesis.
EFFECTS OF IL-3 AND GM-CSF ON AMT

Fig 2. Peripheral blood platelet counts in patients B and C before and during treatment with IL-3 or GM-CSF. In cycles 1 and 3 IL-3 was administered at 62.5 \( \mu g/mol/L^2 \) twice daily SC and at 125 \( \mu g/mol/L^2 \) twice daily SC in cycle 2. GM-CSF was administered at 250 \( \mu g/mol/L^2 \) everyday SC.

10^9/L (range 0 to .62) during cycle 1 and .48 \( \times 10^9/L \) (range .22 to 2.0) during cycle 2. Increases in reticulocyte count and in mean corpuscular volume (MCV) were also observed (change from baseline to peak values shown in Fig 4). These changes were not substantial enough to impact hematocrit.

Following completion of cycle 2, the IL-3 dose was decreased to 125 \( \mu g/m^2/d \) for 7 days and was followed by GM-CSF at 250 \( \mu g/m^2/d \) for 14 days. Cycle 3 failed to result in further increases in thrombopoiesis (Table 3). As seen in the right hand panel of Fig 2, the elevated platelet counts of patients B and C returned to near pretreatment levels. In addition, the interval between patient E's platelet transfusions, which had lengthened during the first two cycles, returned to baseline, and patient A developed a significant conjunctival hemorrhage, her first since starting treatment.

Four of the five patients exhibited a unique pattern of ANC fluctuation during cycle 3. Shortly after initiating GM-CSF, their ANC increased sharply from a median of 2.6 \( \times 10^9/L \) (range 1.2 to 2.8) to a median of 5.6 \( \times 10^9/L \) (range 3.0 to 9.0). The ANC then decreased abruptly (to a median of 1.2 \( \times 10^9/L \), range 0.2 to 6.3) and finally returned to levels greater than those seen on IL-3 by completion of the GM-CSF course. Eosinophil levels did not change consistently during GM-CSF compared with IL-3 treatment (median peak value .48 \( \times 10^9/L \) range .31 to 1.5). While three patients reached their peak reticulocyte count during cycles 1 and 2 and two did
so in cycle 3, MCV increased progressively over time in responding patients.

To further characterize the effects of IL-3 and/or GM-CSF on hematopoiesis, the following studies were also conducted. Bone marrow aspirates and biopsies were obtained in all patients before study inception and at the end of cycle 3. No sample was obtained at the end of IL-3 therapy (completion of cycles 1, 2, and the week of IL-3 in cycle 3). In general, marrows assessed at treatment completion showed minimal to moderate increases in overall cellularity and myeloid:erythroid ratio (Table 4). Megakaryocytes remained so scarce that accurate quantitation was not possible. In an attempt to quantify the peripheral blood stem cell population, the number of CD34 cell surface antigen positive cells was assessed at study entry and following study completion. No difference could be detected by flow cytometry as ±1% CD33+, CD34+ or dual CD33+/CD34+ cells were present both before and after completion of treatment.

Post-study treatment with IL-3. All five patients went on to receive IL-3 at 125 to 250 μg/m²/d for varying intervals after completion of the protocol. Patient A continued to have modest decreased bleeding and platelet transfusion requirement without a documented increase in platelet count for several months before she was lost to follow-up. Patient B, who had initially responded with an increase in platelet count, again responded to IL-3 with a rise in platelets. When IL-3 was discontinued after 1.5 months, platelet counts higher than her pretreatment level were sustained for approximately 40 days without further cytokine administration. The patient's counts then fell and failed to respond to reinstitution of IL-3. Patient C also responded once again with an increase in platelet count on restarting IL-3 but gradually returned to pretreatment baseline after 1 month in spite of ongoing drug administration and manipulation of the dose and schedule of IL-3. In contrast to the lack of measurable platelet increment to IL-3 during cycles 1 and 2, over 9 months of daily administration of IL-3 to patient D has resulted in a slow rise in platelet count from 4 to 6 × 10⁹/L pre-transfusion study to 12 to 18 × 10⁹/L independent of transfusion.

Similarly, patient E had evidence of increased platelet production 2 to 3 weeks into her post-protocol course, became transfusion free 4 months after starting IL-3, and has maintained platelet counts of 15 to 75 × 10⁹/L for 3 months.

Toxicity: Minimal toxicity was observed with either agent or schedule. Early in cycle 1, four of the five patients had low-grade fever responsive to anti-pyretics and subsequently resolved. One patient had recurrence of fever with IL-3 dose escalation (cycle 2). One patient developed urticaria at the IL-3 injection site during cycles 1 and 2, and one had localized urticaria with both IL-3 and GM-CSF (all three cycles). The skin lesions were small and resolved either spontaneously or in the setting of oral diphenhydramine. Additionally, the patients' parents reproducibly reported that their children were more moody and irritable while on IL-3 than previously and that this differed from their behavior while receiving GM-CSF. Given the young patient age as well as change in daily routine, living situation and introduction of sc drug injection twice daily, these observations were difficult to evaluate. No abnormalities were detected in hepatic or renal function on analysis of serial serum samples. No new toxicities have been detected during continued treatment with IL-3.

DISCUSSION

The objectives of this study were twofold. First, we undertook an in vitro evaluation of the AMT defect by assessing the capacity of marrow cells isolated from five patients to generate CFU-MK colonies under both cytokine-stimulated and unstimulated conditions. These experiments suggested that the defect(s) producing the AMT phenotype could be partially corrected by IL-3, GM-CSF, or both. Second, a phase I/II study was undertaken to evaluate the clinical response to administration of sequential courses of IL-3 and/or GM-CSF in these identical five patients. Here, we observed in vivo efficacy of IL-3 but not GM-CSF. No significant toxicity was observed with either schedule although potential increases in moodiness and irritability were of concern as IL-3 receptors are present in the central nervous system.20 Moreover, despite the propensity of marrow failure syndromes to progress to frank myelodysplasia or myeloid leukemia and the potential of hematopoietins to increase this risk,19 no evidence of clonal hematopoiesis was observed in this study before or following cytokine administration. The defect in AMT remains poorly understood. The in vitro studies presented here, although they do not resolve the nature of the deficit, suggest that the clinical phenotype of congenital AMT may result from several different defects in thrombopoiesis. Four of five AMT marrows cultured without added cytokine yielded decreased CFU-MK-derived colonies when compared with normal marrows cultured under the same conditions (P < .05). CFU-MK generation was improved in these four patients by addition of IL-3 and/or GM-CSF in vitro, albeit not to levels observed in normals (P < .05). Moreover, the additive effect of IL-3 and GM-CSF was observed in cultures of normal marrow was observed in only three of five AMT patients. Further, one of five patients exhibited markedly increased CFU-MK production under both unstimulated and cytokine stimulated conditions. Neither in vitro responsiveness nor the magnitude of in vitro response to these cytokines was predictive of their in vivo response. While both IL-3 and GM-CSF induced CFU-MK colony formation in vitro in all patients, only IL-3 induced detectable thrombopoiesis in vivo. The modest and inconsistent level of response argues that defects in AMT are unlikely to result from an isolated defect in IL-3 responsivity of CFU-MK progenitor cells. However, the ability of IL-3 alone to improve thrombopoiesis implies that cytokines required for platelet generation remain active in AMT.

Table 4. Results of Bone Marrow Examination Before and After IL-3 and GM-CSF Treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Marrow Cellularity (%)</th>
<th>Myeloid:Erythroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20–30</td>
<td>1:1.5</td>
</tr>
<tr>
<td>B</td>
<td>40</td>
<td>2:1</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>1:2</td>
</tr>
<tr>
<td>D</td>
<td>55–60</td>
<td>2:1</td>
</tr>
<tr>
<td>E</td>
<td>25–30</td>
<td>1:1.5</td>
</tr>
</tbody>
</table>

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Quantification of clinical response was thus more difficult than expected. All AMT patients demonstrated at least short-term improvement in bleeding diathesis, transfusion requirement, or platelet count during administration of IL-3. Although improvement appeared to be dependent on IL-3 administration, a dose-response relationship was not observed in the range of 125 to 250 ng/mL/d. While two patients clearly demonstrated an increment in platelet count during IL-3 treatment, only improvements in bruising, bleeding, and transfusion requirement could be used to document response in the remaining three patients. After re-institution of IL-3 administration following cycle 3, two of these three patients experienced measurable increases in platelet counts suggesting that more prolonged administration of IL-3 may be necessary to achieve significant platelet increments in some patients. The addition of GM-CSF to IL-3 in cycle 3, at the present dose and schedule, did not lead to further improvement in thrombocytopenia. Rather, platelet production returned to pretreatment levels. These observations further underscore how little is known about adequate duration, schedule, and dose of cytokine therapy in terms of achieving and assessing therapeutic efficacy.

Pre-treatment clinical characteristics may also influence the efficacy of cytokine treatment in AMT patients. Patients B and C, who responded early to IL-3 with rising platelet counts, had the highest initial peripheral counts (Table 1), least bleeding and bruising (Tables 2 and 3), and no chronic platelet transfusion requirement. Therefore, better hematologic function as manifested by preserved peripheral counts and less platelet demand may impact on the degree to which these cytokines can induce a response. Conversely, failure of the remaining three patients to achieve measurable improvement in platelet count during the study may be related to relatively lower initial blood counts (Tables 1 and 3). In addition, ongoing platelet consumption due to bruising and bleeding could have masked our ability to quantitate increased platelet production in these patients (Table 3). These observations suggest that treatment of AMT patients earlier in their disease course might result in more dramatic clinical responses.

Given the progression of AMT to pancytopenia (already evident in these patients), effects on myelopoiesis and erythropoiesis were also measured. In vitro, IL-3 and GM-CSF in combination are more effective than either agent alone in supporting proliferation and differentiation of hematopoietic marrow cells, including CFU-MK. Moreover, non-human primate studies demonstrate that IL-3 administration in vivo can potentiate the effect of subsequent GM-CSF on WBC, reticulocytes, and platelets. In the present study, IL-3 elicited a multilineage response in AMT as demonstrated by increments in platelets, WBC, ANC, reticulocytes, and/or MCV. However, in contrast to previous studies of sequential IL-3 and GM-CSF administration to cancer patients, we observed no increase in CD34+ peripheral blood stem cells after cycle 3. This may reflect important differences both in the frequency of circulating CD34+ cells in patients with marrow failure and/or the potential for recruiting such cells into the circulation. In addition, within the limits of the current trial design, IL-3 pretreatment did not reliably potentiate leukocyte, neutrophil, or reticulocyte response to GM-CSF. In fact, administration of GM-CSF during cycle 3 was associated with an unexpected, oscillatory pattern of ANC in four of five patients. Prolonged exposure of marrow myeloid progenitors to IL-3 followed by the differentiation factor, GM-CSF, likely resulted in the rapid rise, fall, and subsequent recovery of ANC observed. The alternative sequence of GM-CSF exposure followed by IL-3 exposure was delivered to patients restarted on IL-3 after GM-CSF and resulted in an ANC identical to their previous IL-3-stimulated levels (data not shown). This result, also obtained by Donahue et al in subhuman primates, is consistent with the concept of an ordered response to IL-3 and GM-CSF.

Our in vitro studies suggest that IL-3 and GM-CSF used concurrently might be more effective in AMT patients than the sequential design evaluated in this study. Recent analyses of mechanisms of GM-CSF and IL-3 signal transduction through distinct alpha but common beta chains also suggest that the combination of IL-3 and GM-CSF may be much more efficient than either alone. The administration of IL-3 and GM-CSF simultaneously or as a fusion protein might therefore result in increased clinical efficacy. Alternatively, newer agents such as Steel factor or IL-11 may be effective alone or in combination with IL-3. In fact, preliminary in vitro studies show synergy between IL-3 and Steel factor in AMT (Hoffman et al, manuscript in preparation). The optimal choice of cytokine, whether single or in combination, dose, and duration of therapy to best benefit patients with AMT still remains to be established.

ACKNOWLEDGMENT

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