Effects of Recombinant Human Interleukin-3 on Human Hematopoietic Progenitor and Precursor Cells In Vivo

By Oliver G. Ottmann, Arnold Ganser, Gernot Seipelt, Matthias Eder, Gregor Schulz, and Dieter Hoelzer

DNA-synthesis rates and concentrations of bone marrow (BM) and peripheral blood (PB) progenitor cells were studied in 22 patients treated with recombinant human interleukin-3 (rhIL3) as part of a clinical phase I/II study. Recombinant hIL3 at doses of 60 to 500 μg/m² was administered by subcutaneous bolus injection for 15 days to 13 patients with solid tumors and preserved hematopoietic function and to nine patients with bone marrow failure, including five with myelodysplastic syndromes. Following treatment with rhIL3, the percentage of actively cycling BM erythroid (BFU-E) and multilineage (CFU-GEMM) progenitors in patients with preserved hematopoietic function increased from 18% to 36% (P < .06) and from 10% to 40% (P < .01), respectively. The DNA-synthesis rates of early and late granulocyte macrophage progenitor cells increased from 11% to 26% (CFU-GM day 14; P < .02) and from 13% to 30% (CFU-GM day 7; P < .05). There was an increase in BM cellularity from 37% to 58%, and of the myeloid to erythroid ratio from 1.4 to 3.2, while the concentration of marrow progenitors on a per cell basis was unchanged or slightly decreased. The frequencies of immature progenitor cells or continuing immune-mediated suppression. Current evidence suggests that the T-cell-derived lymphokine IL3 stimulates proliferation and differentiation of ontogenetically earlier stem and progenitor cells than the other currently known CSFs. In order to assess the pharmacokinetics, hematologic effects and toxicity of recombinant human IL3 (rhIL3) and its effects on hematopoietic progenitor populations, we recently initiated a phase I/II clinical trial of rhIL3 in patients with solid tumors or bone marrow failure.28 In the present report, we demonstrate the changes in cycling status and concentration of marrow and blood progenitors and the associated changes in bone marrow and hematologic parameters in 22 patients receiving rhIL3 during a 15 day treatment cycle.

INTERLEUKIN 3 (IL3) belongs to a group of hematopoietic colony-stimulating factors (CSFs) initially found to support the growth of hematopoietic progenitors as well as the functional activity of terminally differentiated cells in vitro.1 Corresponding hematopoietic effects in vivo were subsequently demonstrated in preclinical animal models, implying a potential clinical utility of these growth factors in the treatment of hematopoietic failure or during increased hematopoietic demand.2-4 Support for this prediction stems from several recent and ongoing clinical trials that indicate that biosynthetically produced (recombinant) granulocyte-CSF (G-CSF) and granulocyte-macrophage CSF (GM-CSF) are active stimulators of hematopoiesis in humans,5,12-14 and may be effective in mitigating chemotherapy-induced leukopenia15-18 or ameliorating cytopenias in bone marrow failure of diverse etiology, eg congenital and acquired neutropenia,19,20 myelodysplastic syndromes,21,24 and aplastic anemia.22-23 However, CSFs such as GM-CSF have not been universally effective in stimulating hematopoiesis, eg in congenital agranulocytosis,26 severe aplastic anemia or MDS,25,27 possibly due to a lack of activity on sufficiently
HEMATOPOIETIC EFFECTS OF INTERLEUKIN-3 IN VIVO

WHOM BONE MARROW MORPHOLOGY WAS NOT AVAILABLE.

AND 3.5 WEEKS FOLLOWING INTERFERON-Α TREATMENT (3

ANEMIA AND MDS, RESPECTIVELY.

HUMAN PERIPHERAL BLOOD LYMPHOCYTES AND EXPRESSED IN YEAST AS

AG (SEATTLE, WA/MARBURG, FRG). ITS CDNA WAS CLONED FROM

PREVIOUSLY DEScribed.29 THE SECRETED AND PURIFIED MOLECULE HAD A

MOLECULAR WEIGHT OF 14 TO 16 KD AND A SPECIFIC ACTIVITY OF APPROX-

MOTOROUS Bone MARROW AS JUDGED BY A BONE MARROW ASPIRATE AND/OR

NORMAL BONE MARROW MORPHOLOGY AS JUDGED BY A BONE MARROW ASPIRATE AND/OR BIOPSY (N

5FU 5-FLUOROURACIL, CTX CYTOXAN, BLEO BLEOMYCIN.

PACIENTS WITH PRIMARY BONE MARROW FAILURE

14 F/25 CYCLIC THROMBOCYTOPENIA Steroids, lithium 3 60

15 M/33 M. HOECHT INI, SECONDARY BM FAILURE Total nodal irradiation, VCR, PRO, ADR, BLEO, CTX, steroids 56 60

16 M/74 CENTROCYTIC LYMPHOMA, SECONDARY BM FAILURE VBL, PRO, steroids, Cbl, 4-EPI 36 60

17 F/46 BREAST CANCER, BM INFILTRATION 4-EPI, megestrol acetate, aminoglutethimide 30 250

PATIENTS WITH HEMATOPOIETIC INSUFFICIENCY AND/OR TUMOR INFILTRATION OF BONE MARROW

14 F/25 CYCLIC THROMBOCYTOPENIA Steroids, lithium 3 60

15 M/33 M. HOECHT INI, SECONDARY BM FAILURE Total nodal irradiation, VCR, PRO, ADR, BLEO, CTX, steroids 56 60

16 M/74 CENTROCYTIC LYMPHOMA, SECONDARY BM FAILURE VBL, PRO, steroids, Cbl, 4-EPI 36 60

17 F/46 BREAST CANCER, BM INFILTRATION 4-EPI, megestrol acetate, aminoglutethimide 30 250

BETWEEN THE LAST PRECEDING SPECIFIC ANTEINEOPLASTIC THERAPY AND RHIL3

ADMINISTRATION WAS 16 WEEKS (RANGE 2.5 TO 36 WEEKS). TREATMENT

WITH RHIL3 WAS INITIATED 2.5 WEEKS AFTER COMPLETION OF LITHIUM

THERAPY IN ONE PATIENT WITH CYCLIC THROMBOCYTOPENIA, 2.5 WEEKS AFTER

5-FLUOROURACIL (5FU) TREATMENT IN ONE PATIENT WITH PANCREATIC

CARCINOMA WHOSE WBC HAD REMAINED UNCHANGED FOLLOWING 5FU,

AND 3.5 WEEKS FOLLOWING INTERFERON-Α TREATMENT (3 x 10⁶ UNITS THREE TIMES

PER WEEK) IN A PATIENT WITH 5Q- SYNDROME. THREE PATIENTS WITH

NORMAL HEMATOPOIETIC FUNCTION AND THREE PATIENTS WITH MDS HAD

RECEIVED NO PREVIOUS CYTOKINE OR CHEMOTHERAPY. RECOMBINANT Hu-

MAN interleukin 3 was administered by daily subcutaneous bolus

INJECTION AT DOES RANGING FROM 60 TO 500 µG/M2 FOR 15 DAYS. FURTHER

DOSE ESCALATION WAS STOPPED AT 500 µG/M2 BECAUSE OF TOXICITY

THAT CONSIDERED UNACCEPTABLE FOR PROLONGED THERAPY (BONE PAIN, CHILLS AND

FEVER, WHOE GRADE IV) IN TWO PATIENTS (NOT INCLUDED IN THIS REPORT)

AND INCREASED THROMBOCYTOPENIA IN TWO PATIENTS WITH APLASTIC

ANEMIA AND MDS, RESPECTIVELY.

RECOMBINANT IL3 AND OTHER COLONY STIMULATING FACTORS.

RECOMBINANT HUMAN IL3 WAS PROVIDED BY IMMUNEUX/BEHRINGWERKE

AG (SEATTLE, WA/MARBURG, FRG). ITS CDNA WAS CLONED FROM

HUMAN Peripheral BLOOD LYMPHOCYTES AND EXPRESSED IN YEAST AS

PREVIOUSLY DEScribed.29 THE SECRETED AND PURIFIED MOLECULE HAD A

MOLECULAR WEIGHT OF 14 TO 16 KD AND A SPECIFIC ACTIVITY OF APPROX-

IMATELY 1 x 10⁶ UNITS/MG PROTEIN AS DETERMINED IN A BONE MARROW

PROLIFERATION ASSAY. THE CLINICAL PREPARATION WAS FORMULATED IN

LYOPHILIZED FORM TO BE RECONSTITUTED WITH STERILE WATER. ENDOTOXIN

CONTENT WAS <10PG/MG PROTEIN IN THE LIMULUS AMEBOCYTE LYSATE

ASSAY. STERILITY, GENERAL SAFETY AND PURITY STUDIES MET THE FOOD AND

DRUG ADMINISTRATION (FDA) STANDARDS. RECOMBINANT HUMAN G-CSF

AND GM-CSF USED IN THE IN VITRO ASSAYS WAS PURIFIED BY IMMUNEUX

CORP (SEATTLE, WA) AS DESCRIBED29 AND KINDLY PROVIDED BY DR

KRUMWIEH (BEHRINGWERKE AG, MARBURG, FRG). RECOMBINANT Hu-

MAN ERYTHROPOIETIN (EPO) WAS A GENEROUS GIFT FROM BOHRINGER

MANNHEIM AG, MANNHEIM, FRG.

BLOOD AND BONE MARROW SAMPLES AND CELL PREPARATION. PERIPHERAL

BLOOD WAS COLLECTED WITHIN ONE DAY PRIOR TO RHIL3 ADMINISTRATION,

AFTER SEVEN DAYS OF TREATMENT AND ONE DAY AFTER THE LAST DOSE OF RHIL3

(DAY 16). BONE MARROW (BM) ASPIRATES AND TREPHINE BIOPSIES FROM

THE POSTERIOR ILIAC CREST WERE OBTAINED IMMEDIATELY BEFORE AND ON

THE FIRST DAY FOLLOWING THE TREATMENT CYCLE. MONOCLONAL CELLS (MNC)

WERE SEPARATED BY CENTRIFUGATION OVER FICOLL-HYPAQUE AND USED

EITHER DIRECTLY IN PROGENITOR CELL ASSAYS OR CRYOPRESERVED IN 10% FETAL

CALF SERUM (FCS), 10% DMSO AT A CONTROLLED RATE OF 1°C PER

MINUTE USING A CRYOSON PROGRAMMABLE CRYOPRESERVATION APPARATUS.

BONE MARROW MORPHOLOGY. BONE MARROW SMears WERE EVALUATED

AFTER STAINING WITH MAY-GRUNEWALD-GIEMSA (PAPPENHEIM) STAIN.

BIOPSIES WERE DECALCIFIED, AND PARAFFIN-EMBEDDED SECTIONS WERE

STAINED WITH HEATOXELIN-EOSIN, GIEMSA AND GOMORI'S SILVER STAIN

FOR RETICULIN FIBERS. EXAMINATION OF THE ASPIRATES AND BIOPSY SPECI-

MENS WAS PERFORMED UNDER CODE BY TWO INDEPENDENT OBSERVERS. BONE

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marrow cellularity was assessed, by a point counting planimetric method using a 100-point graticule at a magnification of 160x. The relative amounts of hematopoietic tissue, fat cells, bone and vascular structures were calculated from the mean point counts obtained in 15 fields for each biopsy specimen.

*Progenitor cell assays.* Clonogenic assays for burst-forming units-erythroid (BFU-E) and multilineage colony-forming cells (CFU-GEMM) were performed in methylcellulose essentially as described,30 granulocyte-macrophage colony forming cells (CFU-GM) were cultured in 0.3% agar. Low-density bone marrow (LDBM) cells were plated at 5 x 10^5 cells/mL, peripheral blood mononuclear cells at 2 x 10^5 cells/mL in quadruplicate 1 mL aliquots in 35 mm dishes and incubated at 37°C in a fully humidified atmosphere of 5% CO2 in air. Recombinant human GM-CSF, G-CSF and IL3 were incorporated at plateau concentrations (10 ng/mL), previously determined to have maximal colony-stimulating activity in our hands (unpublished observation); EPO (2 U/mL) was added to methylcellulose cultures to facilitate development of BFU-E and CFU-GEMM. Erythroid and mixed colonies were scored on day 14; CFU-GM on days 7 and 14 of culture.

*Calculation of progenitor cell frequencies in blood.* To compare the number of blood progenitor cells per unit volume during treatment with rhIL3, the following equation was used: CFU/mL blood = (CFU/10^6 MNC) x leukocytes/mL blood x (percent MNC in the differential/100).

*Cell cycle analysis.* The tritiated thymidine suicide assay was used as described with slight modifications to determine the percentage of progenitor cells in DNA-synthesis (S-phase). Briefly, LDBM cells were suspended in Iscove's modified Dulbecco medium (IMDM)/10% FCS at 1 x 10^6 cells/mL and duplicate cultures incubated with tritiated thymidine (200 μCi/mL, 40 Ci/mmol/L specific activity, Amersham) for 30 minutes at 37°C. 3H-TdR incorporation was stopped by adding 0.5 mg/mL unlabeled thymidine followed by three washes in ice-cold medium. Control cultures were likewise performed in duplicate. Following pulse treatment with 3H-Tdr or mock, cells from each culture tube were incorporated in clonogenic assays performed in quadruplicate one mL aliquots as described above. For convenience, an arithmetically negative DNA-synthesis rate was considered as zero % progenitors in cell cycle.

*Statistical analysis.* The Wilcoxon rank sum test for paired data and Student’s t-test for paired data were used for statistical analysis.

**RESULTS**

*Cell cycle rates and frequencies of bone marrow progenitors.* The proliferative rate and concentration of BM progenitors were assessed before and immediately following 15 days of treatment with 60 to 500 μg/m2 rhIL3 in nine patients with normal hematopoietic function and no evidence of neoplastic bone marrow involvement; in one patient with cyclic thrombocytopenia; in one patient with centrocytic lymphoma infiltrating the bone marrow; and in one patient with Sβ syndrome (Table 2). The frequency of bone marrow progenitor cells was determined in a tenth patient with intact hematopoiesis in whom too few cells were available for cell cycle analysis. In nine hematopoietically normal patients, the percentage of BFU-E and CFU-GEMM in S-phase (mean ± SD) increased from 16% ± 21% (range 0% to 68%) and 10% ± 18.6% (range 0% to 57%) to 36% ± 12% (range 16% to 56%) and 40% ± 22% (range 0% to 63%), respectively (P < .05 and P < .01, Wilcoxon test). The mean cycling rate of day 14 CFU-GM increased from 10.6 ± 10% (range 0% to 29%) to 26% ± 10% (range 8% to 38%) (P < .02). The DNA-synthesis rate of BFU-E increased in eight and decreased in one patient, while the cycling rates of both CFU-GEMM and day 14 CFU-GM increased in seven of the nine patients and were essentially unchanged in the remaining hematopoietically normal patients (no. 1 and 2 and 1 and 10, respectively) (Table 2). The percentage of actively cycling day 7 CFU-GM increased in five of six patients and was essentially unchanged in one case; mean cycling rates were 13% ± 8% (range 0% to 21%) before and 30.5% ± 8% (range 21% to 39%) after rhIL3 (P < .05, Wilcoxon test). In one patient (no. 1, Table 2) with an exceptionally high percentage of BFU-E, CFU-GEMM and day 14 CFU-GM in DNA-synthesis at baseline (68%, 57% and 29%, respectively), the cycling rate of BFU-E decreased and that of CFU-GEMM and day 14 CFU-GM showed no significant change following treatment with 60 μg/m2 rhIL3. No increase in the cycling rate of erythroid, multilineage or myelomonocytic progenitors was seen in two patients with cyclic thrombocytopenia and bone marrow infiltration by lymphoma, respectively (no. 14 and 16), both of whom received 60 μg rhIL3/m2. In one patient with Sβ syndrome (no. 18), the percentage of early CFU-GM in S-phase increased from 14% pre-treatment to 36% following 15 days of 250 μg rhIL3/m2; CFU-GEMM and BFU-E were undetectable both before and after rhIL3 treatment (Table 2).

In contrast to the cell cycle effects of rhIL3, changes in the frequencies of BM progenitor cells were quite variable even in the group of patients with normal hematopoietic function (Table 2, n = 10): the incidence of BFU-E decreased in six, increased in two, and remained unchanged in two of the ten patients. Their concentration, expressed as colonies (mean ± SD)/1 x 10^7 low-density cells before and after IL3, decreased from 82 ± 52 (range 18 to 179) to 57 ± 27 (range 25 to 105). CFU-GEMM decreased in three, increased in three, and remained essentially unchanged in four of ten patients with a mean (± SD) incidence (1 x 10^7 cells) of 17 ± 17 (range 2 to 53) before and 13 ± 9 (range 3 to 30) after rhIL3. Similarly, the concentration of day 14 CFU-GM decreased in six and increased in four of ten patients; the mean incidence was unchanged at 150 ± 81/1 x 10^5 cells before and 145 ± 75/1 x 10^5 cells after rhIL3. The concentration of day 7 CFU-GM decreased in 5 of seven cases (268 ± 133/1 x 10^5 cells and 159 ± 81/1 x 10^5 cells before and after treatment, respectively). In the majority of cases, the incidence of the different progenitor types changed in a concordant manner; one exception is a patient with normal hematopoiesis (no. 12, Table 2) in whom increases in the concentration of CFU-GEMM and day 14 CFU-GM were accompanied by a decrease in the incidence of BFU-E.

*Bone marrow morphology.* Bone marrow cellularity was quantitated by planimetric analysis of bone marrow biopsies obtained from ten patients (no. 2, 3, 6, 8, 12, 13, 15, 16, 18, 19; Table 1) before and after 15 days of treatment with rhIL3. The mean (±SD) cellularity increased from 37% ± 16% to 58% ± 16% overall (P < .01, Wilcoxon test), and from 36% ± 17% to 64% ± 5% in six patients with normal hematopoiesis (no. 2, 3, 8, 12, 13; Table 1). In five of these patients, the cellularity increased; in one, it remained essentially unchanged (58% and 57%) (no. 6, Table 1). Evaluation of marrow biopsies from four patients with secondary bone...
HEMATOPOIETIC EFFECTS OF INTERLEUKIN-3 IN VIVO

cytops and erythroid precursors decreased from 11.3% to 2% versus 22.2%.

Table 2. Effect of Treatment With rhIL3 on the Incidence and Cell Cycling Rate of BM Progenitors

<table>
<thead>
<tr>
<th>Patient</th>
<th>BM CFU-GM</th>
<th>3H-TdR*</th>
<th>BFU-E</th>
<th>CFU-GEMM</th>
<th>Day 7 CFU-GM</th>
<th>Day 14 CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 60</td>
<td>78 ± 8</td>
<td>25 ± 4</td>
<td>30 ± 2</td>
<td>8 ± 1</td>
<td>463 ± 15</td>
<td>205 ± 18</td>
</tr>
<tr>
<td>+ 25 ± 2 (68)</td>
<td>11 ± 3 (56)</td>
<td>13 ± 5 (67)</td>
<td>3 ± 1 (63)</td>
<td>398 ± 18 (14)</td>
<td>125 ± 8 (39)</td>
<td>228 ± 15 (29)</td>
</tr>
<tr>
<td>2 60</td>
<td>40 ± 5</td>
<td>38 ± 5</td>
<td>4 ± 2</td>
<td>3 ± 2</td>
<td>119 ± 5</td>
<td>125 ± 8</td>
</tr>
<tr>
<td>+ 40 ± 5 (0)</td>
<td>19 ± 5 (50)</td>
<td>4 ± 1 (0)</td>
<td>3 ± 1 (0)</td>
<td>135 ± 10 (0)</td>
<td>78 ± 8 (38)</td>
<td>140 ± 8 (0)</td>
</tr>
<tr>
<td>3 60</td>
<td>99 ± 8</td>
<td>63 ± 2</td>
<td>53 ± 7</td>
<td>17 ± 2</td>
<td>303 ± 15</td>
<td>157 ± 5</td>
</tr>
<tr>
<td>+ ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4 60</td>
<td>95 ± 9</td>
<td>71 ± 7</td>
<td>6 ± 2</td>
<td>8 ± 2</td>
<td>226 ± 6</td>
<td>184 ± 6</td>
</tr>
<tr>
<td>+ 73 ± 8 (23)</td>
<td>46 ± 5 (35)</td>
<td>7 ± 2 (13)</td>
<td>5 ± 1 (38)</td>
<td>184 ± 8 (19)</td>
<td>122 ± 8 (34)</td>
<td>141 ± 8 (13)</td>
</tr>
<tr>
<td>6 125</td>
<td>81 ± 7</td>
<td>78 ± 4</td>
<td>19 ± 2</td>
<td>24 ± 2</td>
<td>203 ± 7</td>
<td>295 ± 16</td>
</tr>
<tr>
<td>+ 65 ± 6 (20)</td>
<td>60 ± 4 (29)</td>
<td>16 ± 2 (16)</td>
<td>10 ± 2 (58)</td>
<td>170 ± 9 (16)</td>
<td>233 ± 6 (21)</td>
<td>118 ± 5 (18)</td>
</tr>
<tr>
<td>7 125</td>
<td>18 ± 4</td>
<td>29 ± 3</td>
<td>2 ± 1</td>
<td>6 ± 2</td>
<td>63 ± 5</td>
<td>114 ± 10</td>
</tr>
<tr>
<td>+ 16 ± 2 (11)</td>
<td>17 ± 3 (41)</td>
<td>2 ± 1 (0)</td>
<td>3 ± 1 (50)</td>
<td>ND</td>
<td>ND</td>
<td>71 ± 8 (0)</td>
</tr>
<tr>
<td>8 250</td>
<td>150 ± 6</td>
<td>75 ± 5</td>
<td>38 ± 4</td>
<td>13 ± 2</td>
<td>205 ± 12</td>
<td>130 ± 11</td>
</tr>
<tr>
<td>+ 130 ± 6 (13)</td>
<td>63 ± 3 (16)</td>
<td>36 ± 4 (6)</td>
<td>7 ± 2 (46)</td>
<td>333 ± 17 (21)</td>
<td>81 ± 5 (23)</td>
<td>208 ± 12 (0)</td>
</tr>
<tr>
<td>9 250</td>
<td>179 ± 7</td>
<td>105 ± 6</td>
<td>6 ± 2</td>
<td>4 ± 1</td>
<td>114 ± 5</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>+ 178 ± 11 (5)</td>
<td>67 ± 7 (36)</td>
<td>7 ± 2 (0)</td>
<td>3 ± 1 (25)</td>
<td>132 ± 8 (6)</td>
<td>29 ± 3 (28)</td>
<td>ND</td>
</tr>
<tr>
<td>10 250</td>
<td>28 ± 2</td>
<td>50 ± 3</td>
<td>3 ± 2</td>
<td>30 ± 4</td>
<td>35 ± 4</td>
<td>195 ± 8</td>
</tr>
<tr>
<td>+ 35 ± 2 (0)</td>
<td>38 ± 3 (24)</td>
<td>3 ± 2 (0)</td>
<td>25 ± 3 (17)</td>
<td>ND</td>
<td>ND</td>
<td>30 ± 2 (14)</td>
</tr>
<tr>
<td>12 500</td>
<td>53 ± 10</td>
<td>33 ± 4</td>
<td>8 ± 2</td>
<td>19 ± 5</td>
<td>198 ± 13</td>
<td>310 ± 10</td>
</tr>
<tr>
<td>+ 49 ± 5 (8)</td>
<td>21 ± 5 (36)</td>
<td>8 ± 1 (0)</td>
<td>7 ± 2 (63)</td>
<td>ND</td>
<td>ND</td>
<td>169 ± 8 (15)</td>
</tr>
</tbody>
</table>

The cell cycle status of BM progenitors was assessed in BM collected within one week before and immediately following 15 full days of rhIL3 administered s.c. at the indicated dosage (µg/m²). The incidence of progenitor cells is given as colonies (mean ± sem) per 1 x 10⁶ LDBM cells. Numbers in parentheses give the percentage of progenitor cells in S-phase of the cell cycle.

Abbreviation: ND, Not Done.

*Pulse exposure of LDBM cells to tritiated or unlabeled thymidine was performed in duplicate cultures, after which the progenitor cell number in each culture tube was assessed in quadruplicate clonal cultures.

marrow failure (no. 13 and 16) and MDS (no. 18 and 19) revealed a 56% ± 9% increase in cellularity above baseline following rhIL3. An enhancement of marrow cellularity by rhIL3 administration was also evident in bone marrow aspirates from nine of eleven patients with normal hematopoietic function (no. 1, 2, 3, 4, 8, 9, 10, 12, 13; Table 1). This resulted primarily from a stimulation of myelopoiesis with a shift to the left and a pronounced eosinophilia. The median myeloid to erythroid ratio was 1.4:1 before and 3.2:1 after treatment. Promyelocytes plus myelocytes increased from 12.7% ± 1.3% to 19.4% ± 1.5% of total marrow cells (P < .01, Wilcoxon test; P < .0025, Student's t-test), and eosinophils increased from 3.9% ± 0.7% to 12% ± 1.3% (P < .01, Wilcoxon test; P < .0005, Student's t-test) (Fig 1). There was no significant change in the percentage of metamyelocytes and bands (23.4% ± 2% versus 22.2% ± 1.4%), segmented neutrophils (12.3% ± 1.8% versus 11.4% ± 2%) or basophils (0.3% ± 0.14% versus 0.5% ± 0.2%). Lymphocytes and erythroid precursors decreased from 11.3% ± 2.2% to 6.8% ± 1.3% (P < .01, Wilcoxon test) and from 32% ± 2.9% to 23.6% ± 2% (P < .05, Wilcoxon test), respectively. Myeloblasts remained undetectable after IL3-treatment, and the percentage of monocytes and plasma cells likewise was essentially unchanged. A similar response pattern was seen in three patients with 5q- syndrome; however, in these patients the rise in eosinophils was accompanied by a conspicuous increase in basophils from 2.3% ± 1% to 8.7% ± 2.7%. The percentage of other mature myeloid elements or of myeloblasts did not change significantly.

Concentration of blood progenitor cells. The frequency of circulating progenitor cell subsets were assessed in maximally stimulated clonogenic assays as described in Methods. Table 3 depicts the results for 12 patients with intact hematopoietic function. Following one week of rhIL3 treatment, day 14 CFU-GM were increased in eight and decreased in three patients with an increase to 200% ± 148% (mean ± SD) of baseline values. CFU-GEMM were increased in seven, decreased in four, and unchanged in one patient with a mean of 172% ± 170% of day 0 levels; BFU-E were elevated above starting levels in three and decreased in nine patients, with a mean of 104% ± 112% of day 0 values. The frequency of circulating progenitor cells detected immediately after completion of rhIL3 treatment was generally lower than after the first week of treatment (Table 3). Numbers of day 14 CFU-GM and CFU-GEMM per volume of blood were 72% and 45% above pre-treatment values.

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respectively, whereas the number of BFU-E decreased by 33%. These changes were not statistically significant. Overall, the interpatient variability of the results was unrelated to the dose level of rHL3.

In three patients with secondary hemopoietic failure and/or tumor infiltration of the bone marrow (no. 15, 16, 17; Table 4), the level of detectable circulating progenitor cells decreased during rHL3 administration, except for an increased number of BFU-E in one patient after one week of treatment. In all five patients with MDS, circulating BFU-E and CFU-GEMM were either absent or present only in extremely low numbers before treatment and were not significantly increased after rHL3 administration (Table 4). CFU-GM increased in three patients with persistence of predominant cluster formation in two of them (no. 20 and 21).

Clinical Response. The WBC increased in 10 of 13 patients after seven days and in all 13 patients with preserved hematopoietic function after 15 days of treatment, from pretreatment leukocyte counts (mean ± SD) of 7,992 ± 3,080/μL (range 3,500/μL to 14,400/μL) to 9,823 ± 2,471/μL (range 5,500/μL to 15,300/μL) after seven days and 13,723 ± 6,821/μL (range 6,500/μL to 25,300/μL) after 15 days of treatment (P < .01, Wilcoxon test). The elevation of the WBC was due to an increase in both neutrophilic granulocytes and eosinophils from pretreatment values of 5,903 ± 2,706/μL and 76 ± 95/μL to 10,335 ± 4,822/μL and 1,988 ± 2,162/μL after 15 days of treatment, respectively (P < .01). The median time interval between initiation of treatment and the maximum response was 14 days for neutrophilic granulocytes and 15 days for eosinophils. Similarly, total leukocytes increased from 5,067 ±

Table 3. Incidence of Blood Progenitor Cells in Patients With Normal Hematopoietic Function During Treatment With IL3

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose level (μg/m²)</th>
<th>Day 14 CFU-GM</th>
<th>Day 15 CFU-GM</th>
<th>BFU-E Day 0</th>
<th>BFU-E Day 7</th>
<th>BFU-E Day 15</th>
<th>CFU-GEMM Day 0</th>
<th>CFU-GEMM Day 7</th>
<th>CFU-GEMM Day 15</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>60 ND</td>
<td>56 (NA)</td>
<td>77 (NA)</td>
<td>377</td>
<td>86 (23)</td>
<td>172 (46)</td>
<td>42</td>
<td>3 (7)</td>
<td>30 (71)</td>
</tr>
<tr>
<td>2</td>
<td>60 ND</td>
<td>91 (79)</td>
<td>ND</td>
<td>316</td>
<td>140 (44)</td>
<td>ND</td>
<td>55</td>
<td>12 (22)</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>60 116</td>
<td>184 (114)</td>
<td>11 (7)</td>
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<td>129 (35)</td>
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<td>130 (25)</td>
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<td>374 (103)</td>
<td>18</td>
<td>20 (111)</td>
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<td>60 58</td>
<td>245 (422)</td>
<td>24 (41)</td>
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<td>262 (423)</td>
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<td>71 (592)</td>
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<td>125 192</td>
<td>388 (202)</td>
<td>59 (31)</td>
<td>550</td>
<td>458 (83)</td>
<td>143 (26)</td>
<td>79</td>
<td>172 (218)</td>
<td>12 (15)</td>
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<td>8</td>
<td>250 66</td>
<td>303 (459)</td>
<td>170 (258)</td>
<td>155</td>
<td>294 (190)</td>
<td>114 (74)</td>
<td>10</td>
<td>39 (390)</td>
<td>14 (140)</td>
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<tr>
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<td>13 (163)</td>
<td>107</td>
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<td>3</td>
<td>2 (67)</td>
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<tr>
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<td>250 40</td>
<td>64 (160)</td>
<td>119 (298)</td>
<td>318</td>
<td>176 (55)</td>
<td>223 (70)</td>
<td>18</td>
<td>30 (166)</td>
<td>22 (122)</td>
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<tr>
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<td>250 276</td>
<td>409 (148)</td>
<td>593 (215)</td>
<td>510</td>
<td>750 (147)</td>
<td>998 (196)</td>
<td>88</td>
<td>167 (190)</td>
<td>624 (709)</td>
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<tr>
<td>12</td>
<td>500 57</td>
<td>191 (335)</td>
<td>73 (128)</td>
<td>113</td>
<td>98 (87)</td>
<td>35 (31)</td>
<td>26</td>
<td>44 (169)</td>
<td>4 (15)</td>
</tr>
<tr>
<td>13</td>
<td>500 253</td>
<td>121 (48)</td>
<td>199 (79)</td>
<td>319</td>
<td>92 (29)</td>
<td>188 (59)</td>
<td>74</td>
<td>21 (28)</td>
<td>17 (23)</td>
</tr>
</tbody>
</table>

Mean ± SD† (100%) (200% ± 148%) (125% ± 106%) (104% ± 112%) (71% ± 50%) (172% ± 170%) (128% ± 207%)

Absolute numbers of CFU-GM, BFU-E and CFU-GEMM/mL blood were determined as described in Materials and Methods. Blood was collected immediately before and after 7 and 15 full days of treatment with IL3 at the indicated dose. SEM was below 12% in all experiments.

Abbreviations: ND, Not Done; NA, Not Applicable.

* Numbers in parentheses give the progenitor incidence as percent of Day 0 values (numbers on Day 0 normalized to 100%).

† Mean ± standard deviation of normalized data.
HEMATOPOIETIC EFFECTS OF INTERLEUKIN-3 IN VIVO

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immediately before and after 7 and 15 full days of treatment with IL3 at the indicated dose. SEM was below 12% in all experiments.

days in three of four patients with altered hematopoietic due to elevated neutrophil and eosinophil levels similar to that rhIL3 would be a broad acting hematopoietic stimulator associated with a significant increase in either hemoglobin or increase in leukocyte counts in the five MDS patients was to 8,740 symptoms being the predominant side effects.

ting its capacity to stimulate progenitor cells growth directly, observed previously following the clinical administration of GM-CSF and GM-CSF and rhIL3 retains its multilineage activity in vivo. Similar effects on the cycling status of BFU-E and CFU-GM have been inferred from the cell cycle data in conjunction with the late-acting hematopoietins or accessory cells. It can be achieved this effect.

Analogous to preclinical and clinical trials of recombinant G-CSF and GM-CSF, the frequencies of bone marrow progenitors were unchanged or reduced following treatment. As dilution of progenitors by the expanded number of more mature erythroid precursors to in vivo stimulation by GM-CSF was reported. In vitro, IL3 and GM-CSF do not support enhanced myelopoiesis to a larger degree than did erythropoiesis as judged by an increased myeloid:erythroid ratio in bone marrow sections and completely failed to enhance hemoglobin levels, hematocrit or erythrocyte numbers. A similar discrepancy between the in vivo effects on erythropoiesis and on myelopoiesis was reported for rhGM-CSF in a recent clinical study, in which a declining responsiveness of more mature erythroid precursors to in vivo stimulation by GM-CSF was reported. In vitro, IL3 and GM-CSF do not support complete hematopoietic differentiation in the absence of late-acting hematopoietins or accessory cells. It can be inferred from the cell cycle data in conjunction with the clinical response to rhIL3 that endogenous late-acting granulopoietic activities are present in sufficient concentrations to result in an elevated total leukocyte count upon in vivo administration of rhIL3 alone, whereas an augmentation of erythrocyte production by rhIL3 requires higher levels of activity supporting terminal erythroid maturation. In a clinical setting, the concomitant administration of erythropoietin and rhIL3 may be the most promising combination to achieve this effect.

Table 4. Incidence of Blood Progenitor Cells in Patients With Hematopoietic Dysfunction During Treatment With IL3

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose level (µg/m²)</th>
<th>Day 0</th>
<th>Day 7*</th>
<th>Day 15</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 15</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 15</th>
</tr>
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<tr>
<td>15</td>
<td>60</td>
<td>3</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3</td>
<td>11 (267)</td>
<td>0 (0)</td>
<td>0</td>
<td>4 (NA)</td>
<td>0 (NA)</td>
</tr>
<tr>
<td>16</td>
<td>60</td>
<td>56</td>
<td>10 (18)</td>
<td>28 (50)</td>
<td>21</td>
<td>14 (67)</td>
<td>11 (52)</td>
<td>8</td>
<td>0 (0)</td>
<td>6 (75)</td>
</tr>
<tr>
<td>17</td>
<td>250</td>
<td>97</td>
<td>42 (43)</td>
<td>40 (41)</td>
<td>366</td>
<td>85 (24)</td>
<td>213 (60)</td>
<td>43</td>
<td>13 (30)</td>
<td>10 (23)</td>
</tr>
<tr>
<td>18</td>
<td>250</td>
<td>12</td>
<td>13 (108)</td>
<td>ND (NA)</td>
<td>0</td>
<td>1 (NA)</td>
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<td>0</td>
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</tr>
<tr>
<td>19</td>
<td>250</td>
<td>0</td>
<td>0 (NA)</td>
<td>0 (NA)</td>
<td>0</td>
<td>0 (NA)</td>
<td>3 (NA)</td>
<td>0</td>
<td>0 (NA)</td>
<td>0 (NA)</td>
</tr>
<tr>
<td>20</td>
<td>500</td>
<td>258</td>
<td>560 (217)</td>
<td>16 (6)</td>
<td>9</td>
<td>0 (0)</td>
<td>2 (22)</td>
<td>2</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>21</td>
<td>500</td>
<td>1939</td>
<td>5682 (292)</td>
<td>5252 (271)</td>
<td>0</td>
<td>0 (NA)</td>
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<td>0</td>
<td>0 (NA)</td>
<td>0 (NA)</td>
</tr>
<tr>
<td>22</td>
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<td>ND</td>
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<td>0</td>
<td>ND</td>
<td>0 (NA)</td>
<td>0</td>
<td>ND</td>
<td>0 (NA)</td>
</tr>
</tbody>
</table>

Absolute numbers of CFU-GM, BFU-E and CFU-GEMM/mL blood were determined as described in Materials and Methods. Blood was collected immediately before and after 7 and 15 full days of treatment with IL3 at the indicated dose. SEM was below 12% in all experiments.

Abbreviations: ND, not done; NA, not applicable.

*Numbers in brackets give the progenitor incidence as percent of day 0 values (numbers on day 0 normalized to 100%).

DISCUSSION

Based on the well-established capacity of interleukin 3 to stimulate multiple hematopoietic lineages in vitro and on initial results from animal studies, it was anticipated that rhIL3 would be a broad acting hematopoietic stimulator in humans. Our evidence that the administration of pharmacologic doses of rhIL3 induces a pronounced increase in the DNA synthesis rate of CFU-GEMM, BFU-E and CFU-GM is in accordance with this expectation and demonstrates that rhIL3 retains its multilineage activity in vivo. Similar effects on the cycling status of BFU-E and CFU-GM have been observed previously following the clinical administration of GM-CSF and EPO. Although IL3 has been shown to act on highly purified progenitor populations in vitro, indicating its capacity to stimulate progenitor cells growth directly, we are not able to discriminate between directly and indirectly mediated hematopoietic effects in vivo. Nevertheless, treatment with rhIL3 was associated with increases in bone marrow cellularity and elevated peripheral WBC counts. The magnitude and the kinetics of the response differed from those observed with G-CSF or GM-CSF and were characterized by a more moderate elevation of leukocyte numbers and a more delayed onset after approximately seven to nine treatment days. These data are consistent with a model in which rhIL3 stimulates hematopoiesis by enhancing stem and progenitor cell proliferation. Despite a comparable stimulation of erythroid, multilineage and myeloid progenitor cell cycling rates, the exogenous administration of rhIL3 resulted in an elevated total leukocyte count upon in vivo stimulation by GM-CSF. In vitro, IL3 and GM-CSF do not support complete hematopoietic differentiation in the absence of late-acting hematopoietins or accessory cells. It can be inferred from the cell cycle data in conjunction with the clinical response to rhIL3 that endogenous late-acting granulopoietic activities are present in sufficient concentrations to result in an elevated total leukocyte count upon in vivo administration of rhIL3 alone, whereas an augmentation of erythrocyte production by rhIL3 requires higher levels of activity supporting terminal erythroid maturation. In a clinical setting, the concomitant administration of erythropoietin and rhIL3 may be the most promising combination to achieve this effect.

Analogous to preclinical and clinical trials of recombinant G-CSF and GM-CSF, the frequencies of bone marrow progenitors were unchanged or reduced following treatment. As dilution of progenitors by the expanded number of more mature precursor and end-stage cells present in the consistently more cellular bone marrow is a likely explanation, the total BM progenitor cell content may well have expanded during treatment. Redistribution of progenitors is another possibility. Except for early CFU-GM and CFU-GEMM,
when assayed after 7 days of rhIL3 treatment, circulating progenitor cell levels were not consistently elevated; rather, the number of BFU-E, and CFU-GEMM after 15 treatment days, per unit volume of blood decreased in the majority of patients, whereas only small increases were observed in the remaining patients. This contrasts with observations from clinical studies of G-CSF, GM-CSF and EPO and a recent study of IL3 in primates, in which considerable elevations of circulating progenitor cells were observed. However, more variable responses during treatment with GM-CSF have also been observed, and murine studies indicate that species differences may account for some of these discrepancies. These may be due to distinct patterns of progenitor cell compartmentalization within different hematopoietic organs during treatment with IL3. These findings raise some doubts that IL3 administration will facilitate procurement of larger quantities of peripheral blood stem cells for use in autologous bone marrow transplantation, as has been suggested for G-CSF and GM-CSF; however, the progenitor cells assayed in this study are known not to be responsive for reconstitution of hematopoiesis. Several details of the changes of cellular composition of the bone marrow during treatment deserve attention. The increased cellularity reflected an increase primarily of promyelocytes, myelocytes and eosinophils; the percentage of blast cells did not increase either in patients with normal bone marrow or those with MDS. In several recent studies, a stimulation of blast cells by GM-CSF was seen predominantly in patients with high initial blast cell counts. Consequently, the latter results can not be extrapolated to MDS in general. An increase of eosinophils and basophils had been anticipated from in vitro studies; in this clinical study a pronounced eosinophilia, but no significant change in basophil numbers, was seen in patients without a primary hematologic disorder. It is presently unclear why elevated basophil counts were seen only in the patients with MDS; possibly, higher levels of basophil maturation activities were present in the patients examined. This lack of a substantial basophilia should in fact be advantageous as a reduced risk of allergic reactions can be anticipated. Indeed, urticarial reactions have been observed in primates treated with IL3. In the present study, no significant allergic reactions were associated with the elevated eosinophil counts. In conclusion, this report demonstrates that rhIL3 has the capacity to enhance hematopoiesis in humans and appears to do so, at least in part, by directly or indirectly stimulating active cycling of multipotential and lineage-restricted progenitor cells. In accordance with its known in vitro and in vivo activities, rhIL3 is likely to have potential clinical utility in bone marrow failure states and should be even more effective in combination with other CSFs acting at later stages of differentiation.

ACKNOWLEDGMENT

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