Recombinant Human Interleukin-3 Expands the Pool of Circulating Hematopoietic Progenitor Cells in Primates—Synergy With Recombinant Human Granulocyte/Macrophage Colony-Stimulating Factor

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The in vivo effect of recombinant human interleukin-3 (rHIL-3) on peripheral blood (PB) levels of hematopoietic progenitor cells was studied in nonhuman primates. Subcutaneous administration of 33 μg/kg/d of rHIL-3 for 11 to 14 days to rhesus monkeys slightly raised leukocyte counts (twofold) and substantially expanded the pool of circulating stem cells in the second week of treatment. At the end of rHIL-3 administration, PB levels of granulocyte/macrophage colony-forming units (CFU-GM) increased by a mean of 12-fold; burst-forming units-erythroid (BFU-E) by ninefold; CFU-mix, by 12-fold; and CFU-megakaryocyte ( Mk), by 13-fold as compared with their respective pretreatment values. Subsequent administration of recombinant human granulocyte/macrophage colony-stimulating factor (rhGM-CSF; 5.5 μg/kg/d for 5 days) to rHIL-3-pretreated animals further expanded the PB stem cell compartment leading to maximum levels of CFU-GM that were in average much more increased (63-fold) than CFU-GM levels under rHIL-3 (14-fold) or rhGM-CSF (12-fold) alone. This hitherto unknown effect of rHIL-3 on the pool of circulating progenitors, particularly in synergy with rhGM-CSF, may facilitate harvest of hematopoietic progenitor cells from PB for stem cell transplantation.

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INTERLEUKIN-3 (IL-3) is one member of a family of glycoproteins that stimulate hematopoietic colony growth in semisolid cultures. The in vitro activities of IL-3 include stimulation of granulocytic (CFU-G), monocytic (CFU-M), granulomonocytic (CFU-GM), eosinophilic (CFU-Eo), megakaryocytic (CFU-Mk), and, in the presence of erythropoietin (EPO), also of erythroid (BFU-E) and multipotential (CFU-mix) colonies from human bone marrow cells. The multilineage effect of this molecule and the fact that IL-3 supports formation of blast cell colonies with high replating capability as well suggest that IL-3 may act early in the hematopoietic cascade. Although IL-3 alone has substantial colony stimulating activity in vitro, optimal stimulation of hematopoiesis requires participation of later acting hematopoietic growth factors, such as granulocyte/macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF).

Recombinant DNA technology has allowed production of sufficient quantities of recombinant human IL-3 (rHIL-3) to permit the use of this molecule in vivo. Initial in vivo studies have demonstrated that rHIL-3 elicited a relatively modest leukocytosis when given to nonhuman primates, but greatly potentiated the responsiveness of the animal to subsequent administration of rhGM-CSF. We report that subcutaneous administration of rHIL-3 to rhesus monkeys substantially increases blood levels of committed and multipotential progenitor cells and that subsequent rhGM-CSF administration synergistically enhances this effect.

MATERIALS AND METHODS

Animals. Ten adult rhesus monkeys, Macaca mulatta, approximately 4 to 8 years old and weighing between 5 and 15 kg, were housed in individual stainless steel cages. Monkeys were provided with 10 changes per hour fresh air, conditioned to 23°C ± 2°C with a relative humidity of 60% ± 10%. They were maintained in a 12 hour light/dark cycle and provided with tap water ad libitum and commercial primate chow and fruit. Research was conducted according to the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources, National Research Council.

Recombinant cytokines. Recombinant human IL-3 used in this study was provided by Genetics Institute, Cambridge, MA. The nonglycosylated protein was extracted from Escherichia coli cells expressing the IL-3 cDNA from a plasmid vector. Recombinant human IL-3 accumulating intracellularly was purified to homogeneity by a series of chromatographic steps, including high-performance liquid chromatography (HPLC). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed the presence of a single Coomassie blue staining band with a purity of ≥95% and a molecular weight of 14 to 15 Kd. The intrinsic biologic activity was found to be 4.6 x 10^6 U/mg protein assayed by thymidine incorporation by chronic myelogenous leukemia (CML) myeloblasts, as described. The endotoxin content was 30 ± 5 pg/mg as determined by the limulus assay. The endotoxin content, 1.4 ± 0.3 ng/mg, was determined by the minum assay. Peripheral blood (PB; 5 mL) was collected into sterile tubes containing 1 mL EDTA. Bone marrow (BM) samples were obtained by aspiration into sterile tubes containing heparin with no preservative (Seromed, Berlin, FRG). BM mononuclear cells (MNC) and BM MNC were harvested after a Ficoll-Hypaque gradient centrifugation (400g for 40 minutes, 1.077 g/mL).

Progenitor cell assays. CFU-GM, BFU-E, CFU-mix, and CFU-Mk progenitor cells were assayed using a modification of the clonal assay described by Fauser and Messner. Each plate contained 0.9% methylcellulose, 30% fetal calf serum (FCS), 10% bovine serum albumin (BSA; Behring, Marburg, FRG), alpha-thioglycolate.

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(10^4 mol/L), 5% phytohemagglutinin-leukocyte-conditioned medium (PHA-LCM), and Iscove's modified Dulbecco's medium (IMDM; GIBCO, Paisley, Scotland). For cultivation of BFU-E and CFU-mix, 1 U/mL erythropoietin (EPO; Toyobo, Osaka, Japan) was added to culture dishes. PB MNC and BM MNC were plated in triplicate at 0.7 to 2.0 x 10^5/mL. After a culture period of 14 days (37°C, 5% CO₂, full humidity), cultures were examined under an inverted microscope. Aggregates with more than 40 translucent, dispersed cells were counted as CFU-GM. Bursts containing more than 100 red colored cells were scored as BFU-E. CFU-mix were identified by their heterogeneous composition of translucent and hemoglobinized cells. All colonies suspected as CFU-mix were picked, transferred to glass slides, and stained with May-Grünwald-Giemsa for cytologic examination under a light microscope. CFU-Mk were cultured as described above except using autologous serum, which was harvested before administration of cytokines, instead of FCS. Aggregates containing at least four large megakaryocytic cells were scored as CFU-Mk.

The total number of MNC per milliliter of PB was determined by multiplying the leukocyte count by the percentage of MNC in the differential. The MNC fraction consists of monocytes, lymphocytes, and immature myeloid forms circulating in the PB. The total numbers of hematopoietic progenitors per milliliter of PB were then determined by multiplying the number of colonies per 10^5 MNC by the total number of MNC per milliliter of PB. BM progenitors were expressed as colonies per 1.0 x 10^5 BM MNC plated.

**Hematologic examinations.** For hematologic examinations, blood was collected in EDTA-coated tubes. Parameters measured included the total counts of red blood cells (RBCs), white blood cells (WBCs), platelets, and determination of hemoglobin and hematocrit (Sysmex 2000; TOA, Tokyo, Japan). Differential blood cell counts were established on the examination of 200 cells of a Giemsa-stained blood smear by two independent observers.

**Administration of rh cytokines to animals.** Recombinant human IL-3 and rhGM-CSF were prepared for subcutaneous (SC) administration by thawing the daily dose and diluting with 6 mL nonpyrogenic saline containing 0.5% monkey serum. Two milliliters of the rhIL-3 or rhGM-CSF solution was given SC three times daily for 11 or 14 days in case of rhIL-3 and for 5, 11, or 14 days in case of rhGM-CSF. Control monkeys received SC injections of nonpyrogenic saline supplemented with 0.5% monkey serum containing the same amount of endotoxin (LPS World Health Organization standard from *E coli* 15) detected in the rhIL-3 and rhGM-CSF preparation, respectively.

**RESULTS**

*Effect of rhIL-3 on CFU-GM levels in PB.* Figure 1 shows mean values from serial determinations of PB CFU-GM levels (A) and of WBC counts (B) over a period of 11 days in five rhesus monkeys receiving 33 μg/kg/d of rhIL-3. Numbers of circulating CFU-GM began to rise after 2 days of rhIL-3 administration and exceeded 1,000/mL in the second week of treatment in all five animals. On the average, this dose of rhIL-3 raised CFU-GM levels by approximately 12-fold from a mean pretreatment level of 274/mL (range, 231 to 344) to a mean level of 3,273/mL (range, 1,217 to 4,771) on day 11 (P < .02 by the Students' *t* test for paired samples). CFU-GM levels in two monkeys receiving 11 μg/kg/d of rhIL-3 for 2 weeks did not exceed 1,000/mL (data not shown), indicating a dose-dependent increase of PB CFU-GM counts by rhIL-3. As compared with the effect of rhIL-3 on CFU-GM counts in PB, the effect of rhIL-3 on mature cells in PB was less pronounced in the same animals.

There was an approximately twofold increase in leukocyte counts from a mean pretreatment value of 13,100/μL (range, 8,300 to 20,000) to 27,000/μL (range, 17,900 to 42,800) on day 11 of rhIL-3 treatment. As reported recently by us, this increment in WBC count is mainly due to elevation of basophils and eosinophils. While most monkeys developed transient urticaria like skin lesions, no other toxicity was seen by clinical or laboratory examination.

*Effect of rhIL-3 on BFU-E, CFU-mix, and CFU-Mk levels in PB.* In four rhesus monkeys treated with 33 μg/kg/d of rhIL-3, levels of circulating erythroid, multipotential, and megakaryocytic progenitors were determined before, on day 7, and immediately after treatment (day 11 or 14). As shown in Table 1, the in vivo effect of rhIL-3 was not restricted to CFU-GM but included a broad spectrum of hematopoietic
rhIL-3 EXPANDS THE PB PROGENITOR CELL POOL

Table 1. Effect of rhIL-3 on Levels of BFU-E, CFU-Mix, and CFU-Mk in the PB From Four Rhesus Monkeys

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Duration of IL-3 (d)</th>
<th>BFU-E/mL PB</th>
<th>CFU-mix/mL PB</th>
<th>CFU-Mk/mL PB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre- IL-3</td>
<td>7 d of IL-3</td>
<td>End of IL-3</td>
<td>Pre- IL-3</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>12</td>
<td>0</td>
<td>143</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
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<td>47</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>74</td>
</tr>
</tbody>
</table>

Group mean (SD) | 9 (11) | 13 (26) | 82 (42) | 4 (9) | 0 (0) | 49 (24) | 48 (55) | 877 (1,210) | 619 (262) |

P > 0.1 P > 0.1 P > 0.1 P < 0.05 P < 0.02 P < 0.05

All four animals received 33 μg/kg/d of rhIL-3 SC in three daily doses. Progenitor cell determinations were done as described in Materials and Methods and are given as means of triplicate cultures before, during, and at the end of rhIL-3 treatment. The Student’s t test for paired samples was used to compare groups.

Effect of sequential administration of rhIL-3 and rhGM-CSF on progenitor cell levels in PB. We have recently shown that rhIL-3 potentiates the increase in WBC counts by subsequent administration of rhGM-CSF in rhesus monkeys but that this synergistic effect is not observed if both cytokines are given simultaneously.4 In Fig 2, progenitor cell data are given from a monkey that was pretreated with rhIL-3 (33 μg/kg/d) for 14 days and subsequently received rhGM-CSF (5.5 μg/kg/d) for only 5 days. After 3 months, the same animal was studied under rhGM-CSF alone in order to evaluate the permissive effect of rhIL-3. Recombinant human GM-CSF massively increased numbers of PB CFU-GM after rhIL-3 pretreatment (A) but was only moderately effective in the same individual if rhIL-3 was not given before (open circles). This boosting effect of rhGM-CSF on progenitor cell counts was also observed, although to a lesser extent, in all other progenitor cell types investigated (B). A rapid (within 2 to 5 days) and massive increment of PB CFU-GM numbers under rhGM-CSF was observed in four of five rhIL-3-pretreated rhesus monkeys, an increment that could not be further enhanced if rhGM-CSF administration was continued for 2 weeks (in one animal, data not shown). CFU-GM levels in animals not pretreated with rhIL-3 but receiving rhGM-CSF for 11 or 14 days showed a more delayed response, and reached their maximum between days 7 and 14. Figure 3 compares maximum levels of circulating CFU-GM that were obtained by administration of either cytokine alone or by the sequential administration of rhIL-3 and rhGM-CSF. Maximum CFU-GM levels were markedly scattered in animals treated sequentially with both cytokines, but were on the average far higher (17,323/mL, 8,000 CFU-GM/mL PB) after sequential administration. For personal use only. on October 30, 2017. For personal use only.
Fig 3. Maximum increases in peripheral blood CFU-GM levels under rhIL-3, rh GM-CSF, or the sequential administration of both cytokines. Five monkeys received 33 μg/kg/d of rhIL-3 for 11 or 14 days and were subsequently treated with 5.5 μg/kg/d of rhGM-CSF for 5 (four animals) or 14 (one animal) days. CFU-GM numbers were determined every 2 to 3 days as described in Materials and Methods. Increases from pretreatment values to maximum values under rhIL-3 (IL-3) and subsequent rhGM-CSF administration (IL-3 + GM-CSF) are shown. Maximum increases in CFU-GM levels are also given from three separate monkeys treated with 5.5 μg/kg/d of rhGM-CSF alone (GM-CSF) for 11 or 14 days. Data for all animals represent means from triplicate cultures.

63-fold increase above pretreatment value) than maximum levels under rhIL-3 (3,853/mL, 14-fold increase) or rhGM-CSF (3,232/mL, 12-fold increase) alone.

Effect of rhIL-3 and rhGM-CSF on BM progenitors. In two animals, effects of rhIL-3 treatment and subsequent rhGM-CSF administration on BM progenitors were investigated (Fig 4). No major and consistent effect in progenitor cell concentrations could be observed under rhIL-3 (day 14). In contrast, BM progenitors of all types were consistently decreased under subsequent administration of rhGM-CSF in both animals (day 18), but returned to pretreatment values after termination of rhGM-CSF administration (day 36).

DISCUSSION

The impressive stimulatory effects of recombinant hematopoietic growth factors such as GM-CSF, G-CSF, or EPO in cytopenic patients indicate that a new form of medical therapeutics has evolved. Subcutaneous injections of rhIL-3 to rhesus monkeys clearly produced hematopoietic changes in PB. Recently, we and other investigators demonstrated that rhIL-3 induces moderate leukocytosis in primates and greatly potentiates the increase in WBC counts by subsequent administration of rhGM-CSF.7,8 In this article, we show that rhIL-3 substantially increases blood levels of committed and of multipotential progenitor cells. In general, the hematopoietic changes under rhIL-3 were more pronounced in the progenitor cell compartment than in the compartment of mature blood cells. For example, levels of megakaryocytic progenitors (CFU-Mk) were found to be highly elevated under rhIL-3, whereas platelet counts remained unchanged in the same animals. These in vivo findings seem to support the concept that IL-3, as a multilin-
eage and, therefore, early acting hematopoietic growth factor, mainly affects the stem cell compartment, but later acting growth factors may be required to generate mature blood cells from committed progenitors.

This in vivo study does not allow us to conclude how the effect of rhIL-3 and rhGM-CSF alone or in combination on the progenitor cell compartment is being mediated. In vitro, IL-3 and GM-CSF are effective stimulators of hematopoietic colony formation from highly enriched human BM cells, suggesting direct actions of these molecules on hematopoietic progenitors.24 On the other hand, GM-CSF has been shown to induce release of tumor necrosis factor-α (TNF-α) from human monocytes,25 which could subsequently trigger the release of CSFs or other intermediary molecules from accessory cells. Paquette et al11 have demonstrated a synergistic stimulation of colony formation in semisolid cultures by the combination of IL-3 and GM-CSF, and others have shown a synergistic action of both molecules on the leukocyte counts in primates if given sequentially.7,8 Although rhIL-3 and rhGM-CSF substantially expanded the compartment of circulating progenitors, we do not know their effect on the size of the total stem cell compartment. Progenitor cell data from BM must be considered with caution because of the possibility of a variable dilution of BM aspirates by PB. However, the rapid increase in levels of PB progenitors under rhGM-CSF in face of a decrease in concentration of BM progenitor cells suggests some redistribution effect in case of rhGM-CSF.

Regardless of the mechanism involved, rhIL-3 may be useful to raise levels of circulating hematopoietic progenitors. An increase of blood CFU-GM levels has been reported in cancer patients treated with rhGM-CSF19 or rhG-CSF.20 With the sequential administration of rhIL-3 and rhGM-CSF, we achieved an increment in progenitor cell numbers in blood of approximately 60-fold as compared with baseline values. This increment was on the average much higher than that obtained with either factor alone. We do not know if the sequential application of rhIL-3 and rhGM-CSF is the optimal combination to boost circulating progenitors to maximal levels or if other combinations of recombinant hematopoietic growth factors containing or not containing rhIL-3 are similar or even more effective.

Circulating stem cells are able to reconstitute hematopoiesis after supralethal chemotherapy and/or irradiation.21 In man, a factor limiting PB transplantation is the inability to obtain sufficient quantities of progenitor cells from the PB in a reasonable period of time. The number of circulating progenitors may rise considerably during recovery from cytotoxic-induced BM aplasia. This phenomenon, first demonstrated in dogs,22 also occurs in man23 and can be exploited to collect a sufficient number of circulating stem cells for autografting if several leukapheresis sessions are done during the rebound period after chemotherapy.24,25 The effect of rhIL-3, particularly in combination with rhGM-CSF, on the compartment of circulating progenitor cells may help to reduce the number of leukapheresis sessions required or even permit collection of a sufficient amount of stem cells from PB without preceeding chemotherapy.

The progenitor cells we have assayed in this study might be important for an early but limited engraftment phase26 and may, therefore, be clinically useful in shortening the time of aplasia after myeloablative therapy. However, only grafting studies can clarify whether the stem cell pool expanded by recombinant cytokines also contains most primitive progenitors that are able to restore hematopoiesis persistently.

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**REFERENCES**

12. Fauser AA, Messner HA: Identification of megakaryocytes,
Macrophages, and eosinophils in colonies of human bone marrow containing neutrophilic granulocytes and erythroblasts. Blood 53:1023, 1979


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