Highly Increased Production of Bone Marrow-Derived Blood Cells by Administration of Homologous Interleukin-3 to Rhesus Monkeys

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Recombinant rhesus monkey interleukin-3 (IL-3) was administered to normal rhesus monkeys in graded doses ranging from 3 to 30 μg/kg/d subcutaneously for 30 consecutive days or given as a continuous intravenous infusion at a dose of 30 μg/kg/d for 16 days. After a lag phase of about 1 week, a highly increased, dose-dependent production of bone marrow-derived blood cells was observed, preceded by amplification of bone marrow hematopoietic progenitor cells. Simultaneously, peripheral blood progenitor cells rose. The increases included basophilic, eosinophilic and neutrophilic granulocytes, monocytes, and the erythrocyte and platelet lineages. Characteristically, a T-lymphocyte response was absent. It is concluded that IL-3 in vivo stimulates blood cell production from an immature, multipotent progenitor cell.

INTERLEUKIN-3 (IL-3), also termed multilineage colony-stimulating factor (CSF), is a cytokine involved in bone cell formation. IL-3 stimulates in vitro a bone marrow cell population ancestral to most, if not all, of the bone marrow-derived blood cells, in addition to pre-B cells, mast cells, natural cytotoxic cells, the formation of osteoclasts, blast cells in acute myeloid leukemia, but not prothymocytes or natural killer cells. Before the identification and naming of murine IL-3, its biologic activity has been apparent from stimulation of murine stem cells. Contrasting its broad range of action in vitro, recombinant human IL-3, administered to rhesus monkeys (Macaca mulatta) and cebus monkeys (Macaca fascicularis), exerted limited and in part inconsistent effects on blood cell production. Somewhat larger effects of human IL-3 on peripheral blood numbers were noted by sequential administration of another hematopoietic growth factor, granulocyte-macrophage CSF (GM-CSF). Therefore, it is generally held that IL-3 expands an early cell population that then requires the action of a later acting factor such as GM-CSF to complete its development. As an alternative hypothesis we proposed the limited effects of human IL-3 in Macaca species to be in part attributable to its species specificity. Hence, we isolated the gene encoding rhesus monkey IL-3 (Rh-IL-3),22 The Rh-IL-3 gene encodes a mature protein of 124 amino acids that lacks 9 C-terminal amino acids of human IL-3 and differs in 23 amino acids from the remaining mature human IL-3. Comparison of the coding DNA sequences of Rh-IL-3 with those of mice, rats, gibbons, and humans showed a high rate of nonsynonymous nucleotide substitutions, which provides an explanation for the species specificity encountered for IL-3. Here we report the multilineage effects of homologous IL-3 in rhesus monkeys.

MATERIALS AND METHODS

Monkeys. Young adult male monkeys (Macaca mulatta) with a body weight of 3 to 4 kg were used throughout this study.

IL-3. Rh-IL-3, expressed and excreted by B. licheniformis, was purified to homogeneity as described. Its specific activity as tested on the basis of colony formation by purified Rhesus monkey bone marrow cells was about 10^6 U/mg protein.

IL-3 infusion. For the monkey receiving IL-3 intravenously (IV), a small miniature infusion pump (Dahedi Elektroniks, Maarsen, Holland) was connected to a Port-a-Cath system 527 CS (Pharmacia) subcutaneously implanted and entering a jugular vein.
RESULTS
Rh-IL-3 was administered to rhesus monkeys in doses ranging from 3 to 30 \( \mu \text{g/kg/d} \) subcutaneously during 30 consecutive days to test its in vivo effects. One monkey received a continuous IV infusion at the highest dose used as a pilot experiment for comparison of subcutaneous versus IV routes of administration, which was discontinued because of severity of side effects after 16 consecutive days. After a lag phase of 1 week, a strong dose-dependent effect on the numbers of nucleated blood cells, including normoblasts, was noted (Fig 1). Analysis of WBCs showed increases in numbers of eosinophilic and neutrophilic granulocytes and the appearance of large numbers of cells designated as atypical (basophilic) granulocytes, reported earlier.\textsuperscript{19,21} Accordingly, intracellular histamine levels of peripheral blood cells increased directly proportional to the numbers of these atypical basophils (Table 1 and Fig 1). Monocytes and lymphocytes increased in number. Highest WBC counts (up to \( 75 \times 10^9/L \)) were observed in the monkey infused with 30 \( \mu \text{g/kg/d} \). Neither absolute cell numbers nor the variety of cell types produced are preceded in studies with human IL-3 in \textit{Macaca} species.\textsuperscript{19-21}

Peripheral blood cells were also monitored by measuring the frequency of cells with the myeloid differentiation antigen CD11b versus the number of T lymphocytes as characterized by CD4/CD8 antigens. Together these markers identify the vast majority of WBCs. CD11b positive cells, including atypical basophilic granulocytes, showed an IL-3 dose-dependent increase, whereas the T-lymphocyte numbers were not appreciably influenced by IL-3. In two monkeys that received 3 or 10 \( \mu \text{g/kg/d} \), respectively, T lymphocytes were measured every 3 days during IL-3 treatment; peripheral blood T cells were stable at a mean value of \( 3.3 \pm 1.5 \times 10^9/L \), not different from simultaneously determined normal values.

The RBC lineage was strongly stimulated by IL-3. More than sixfold increases of reticulocyte numbers were observed in the monkeys that received 10 or 30 \( \mu \text{g/kg/d} \) (Table 1). Normoblast numbers rose to \( 10^9/L \) in the recipient of 30 \( \mu \text{g/kg/d} \) subcutaneously and up to \( 18 \times 10^9/L \) in the monkey that received a continuous infusion of 30 \( \mu \text{g/kg/d} \) (Fig 1). The reticulocytosis did not translate into an increase in RBC numbers, most likely due to the frequent blood and bone marrow punctures for analyses. In addition, vast numbers of circulating normoblasts may also point to ineffective erythropoiesis, suggesting a possible lack of erythropoietin in levels proportional to those of IL-3.

Bone marrow was punctured weekly as specified in Table

Fig 1. Peripheral nucleated blood cell counts after administration of graded doses of Rh-IL-3, from top to bottom panels: continuous infusion of 30 \( \mu \text{g/kg/d} \) during 16 consecutive days, and daily single subcutaneous injection of 30, 10, and 3 \( \mu \text{g/kg/d} \), respectively, given for 30 consecutive days and a simultaneous control monkey, which did not receive IL-3, but was otherwise treated identically. The differential counts were cumulated, from top to bottom: normoblasts, eosinophilic and neutrophilic granulocytes, neutrophilic bands, atypical granulocytes, monocytes and lymphocytes. Note scale difference between upper panel and the other panels.
Table 1. Reticulocyte Numbers, Surface Marker Analysis, and Histamine Levels During IL-3 Administration to Rhesus Monkeys in Comparison With Controls

<table>
<thead>
<tr>
<th>IL-3 Dose (µg/kg/d)</th>
<th>Reticulocytes (10⁶/L)</th>
<th>CD11b⁺</th>
<th>CD4⁺/CD8⁺ (10⁶/L)</th>
<th>Histamine (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 8</td>
<td>d 16</td>
<td>d 24</td>
<td>d 37</td>
</tr>
<tr>
<td>0</td>
<td>49</td>
<td>72</td>
<td>61</td>
<td>103</td>
</tr>
<tr>
<td>3 SC</td>
<td>47</td>
<td>73</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>3 SC</td>
<td>131</td>
<td>121</td>
<td>153</td>
<td>76</td>
</tr>
<tr>
<td>10 SC</td>
<td>93</td>
<td>364</td>
<td>420</td>
<td>121</td>
</tr>
<tr>
<td>10 SC</td>
<td>78</td>
<td>239</td>
<td>215</td>
<td>156</td>
</tr>
<tr>
<td>30 SC</td>
<td>84</td>
<td>293</td>
<td>539</td>
<td>193</td>
</tr>
<tr>
<td>30 IV</td>
<td>97</td>
<td>238</td>
<td>234</td>
<td>118</td>
</tr>
</tbody>
</table>

Normal ± SD

<table>
<thead>
<tr>
<th>IL-3 Dose (µg/kg/d)</th>
<th>SC and IV indicate subcutaneous and intravenous routes of administration, respectively. WBC, white blood cells (excluding normoblasts); ND, not done.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-3 Dose (µg/kg/d)</td>
<td>SC and IV indicate subcutaneous and intravenous routes of administration, respectively. WBC, white blood cells (excluding normoblasts); ND, not done.</td>
</tr>
</tbody>
</table>

2. Total punctate cellularity during treatment showed dose-dependent increases after 1 week of IL-3 administration. Dose dependence was lost after 2 weeks when values of 3.8 ± 2.4 x 10⁶ (mean ± SD) nucleated cells/mL punctate were reached as opposed to 0.7 ± 0.6 x 10⁶ cells/mL for pretreatment punctates combined with those of the control monkey. IL-3–stimulated bone marrow cellularity was maintained during the third (1.6 ± 0.5 x 10⁶/mL) and fourth weeks (3.3 ± 2.2 x 10⁶/mL). It decreased to low numbers in the first week after cessation of IL-3 administration (0.2 ± 0.1 x 10⁶/mL), but returned to more normal numbers (0.4 ± 0.2 x 10⁶/mL) in the second week posttreatment.

Table 2. Bone Marrow and Peripheral Blood Progenitor Cells During and After Administration of IL-3 to Rhesus Monkeys

<table>
<thead>
<tr>
<th>IL-3 Dose (µg/kg/d)</th>
<th>Nucleated Cells/mL Bone Marrow Punctate (× 10⁶)</th>
<th>GM-CFU/mL Bone Marrow (× 10⁴)</th>
<th>BFU-E/mL Bone Marrow (× 10⁶)</th>
<th>GM-CFU/mL Blood (× 10⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 7</td>
<td>d 14</td>
<td>d 21</td>
<td>d 28</td>
</tr>
<tr>
<td>3 SC d 0-30</td>
<td>23</td>
<td>686</td>
<td>118</td>
<td>328</td>
</tr>
<tr>
<td>3 SC d 0-30</td>
<td>27</td>
<td>232</td>
<td>217</td>
<td>73</td>
</tr>
<tr>
<td>10 SC d 0-30</td>
<td>60</td>
<td>241</td>
<td>242</td>
<td>553</td>
</tr>
<tr>
<td>10 SC d 0-30</td>
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<td>702</td>
<td>172</td>
<td>104</td>
</tr>
<tr>
<td>30 SC d 0-30</td>
<td>190</td>
<td>163</td>
<td>112</td>
<td>292</td>
</tr>
<tr>
<td>30 IV d 0-16</td>
<td>692</td>
<td>245</td>
<td>120</td>
<td>640</td>
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</tbody>
</table>

Normal value ± SEM

<table>
<thead>
<tr>
<th>IL-3 Dose (µg/kg/d)</th>
<th>Normal value ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 SC d 0-30</td>
<td>73 ± 12</td>
</tr>
<tr>
<td>3 SC d 0-30</td>
<td>131 ± 33</td>
</tr>
<tr>
<td>3 SC d 0-30</td>
<td>46 ± 20</td>
</tr>
<tr>
<td>3 SC d 0-30</td>
<td>6 ± 2</td>
</tr>
</tbody>
</table>

SC, IV, ND, and SEM indicate subcutaneous, intravenous, not done, and standard error of the mean, respectively. IV administration of high-dose IL-3 was discontinued after day 16 because of severity of side effects.
Fig 2. Area of a bone marrow clot preparation of an untreated rhesus monkey (A) and of the rhesus monkey receiving a continuous infusion of 30 μg/kg/d Rh-IL-3 after 2 weeks of treatment (B). Note vastly increased cellularity, disappearance of fat cells, abundant juvenile granulocytes, RBC precursors, and megakaryocytes in the IL-3-treated monkey. Clots were stained with hematoxylin-phloxin-saffron.

Prominent features of bone marrow morphology were dose-dependent increases of undifferentiated cells, atypical basophilic granulocytes, megakaryocytes, and eosinophilic precursors. Juvenile neutrophils as well as erythroid precursor cells in all stages of development were most numerous (Fig 2). The frequency of in vitro detected immature colony-forming hematopoietic progenitor cells GM-CFU and BFU-E increased as well throughout the treatment with IL-3. An illustrative example was provided by the monkey that received Rh-IL-3 IV. In this animal the progenitor cell numbers had increased on the seventh day of IL-3 treatment to $2.5 \times 10^6$ GM-CFU/mL punctate from a pretreatment
number of $16 \times 10^4$ GM-CFU/mL and to $3 \times 10^5$ BFU-E/mL from $8 \times 10^3$ BFU-E/mL. Because peripheral blood counts during the first week of IL-3 administration did not show major changes (Fig 1), it is concluded that IL-3 initiated production of blood cells is preceded by amplification of immature bone marrow hematopoietic progenitor cells. In the same animal, the peripheral blood GM-CFU increased from $10^3/mL$ pretreatment to $0.8 \times 10^7/mL$ after 14 days of IL-3 administration without an appreciable change during the first week.

The thrombocyte response to administration of Rh-IL-3 showed a peculiar dose dependence. At lower doses, a clear thrombocytosis was observed, which lasted for 2 weeks after discontinuation (Fig 3). The monkeys that received $3 \mu g/kg/d$ had mean peak thrombocyte counts of $618 \times 10^9/L$, starting from a mean pretreatment value of $377 \times 10^9/L$, while those receiving $10 \mu g/kg/d$ increased from a mean of $285 \times 10^9/L$ pretreatment to a maximum level of $580 \times 10^9/L$. The monkeys that received IL-3 in a dose of $30 \mu g/kg/d$ developed profound thrombocytopenia. Because all bone marrow preparations showed active megakaryocytopenia (eg, Fig 2) and shift platelets were abundant (data not shown), it was concluded that the thrombocytopenia reflected an increased consumption rather than decreased production.

Dose-related side effects (Table 3) included urticaria starting around day 10 of treatment, prominent axillary and inguinal lymph nodes from day 20 onward, and at high IL-3 doses facial and scrotal edema starting in the third week of treatment. The skin lesions were most numerous and prominent in monkeys that received 30 $\mu g/kg/d$ IL-3. Histologically (Fig 4), skin lesions were characterized by (1) marked perivascular accumulation of neutrophils, eosinophils, and most predominant by toluidine blue staining (not shown), mast cells; (2) widespread endothelial destruction; (3) marked superficial dermal edema; and (4) hemorrhage in most severely affected sites. Thrombocytopenia was coincident with vasculitis and hemorrhage in the generalized skin lesions of the monkeys administered IL-3 in high doses. Withdrawal of IL-3 not only prompted spontaneous resolution of skin lesions, but also of thrombocytopenia and other side effects without any sequela within 2 to 4 days.

**DISCUSSION**

The results presented here demonstrate that in vivo administration of homologous IL-3 to rhesus monkeys resulted in a dose-dependent stimulation of the production of all bone marrow-derived cell lineages. In line with existing in vitro data and strongly supported by bone marrow analyses, this may be simply explained by increased production of progenitor cells from developmentally early, multipotential bone marrow cells resulting in augmented production of all bone marrow-derived blood cell lineages. Characteristically, a peripheral blood T-lymphocyte response was absent. The magnitude of the IL-3 responses described here are comparable with those seen after administration of murine recombinant IL-3 to mice or to the myeloproliferative disorder observed after retrovirus-mediated transfer and expression of the mouse IL-3 gene in mouse hematopoietic cells.

Among the hematopoietic growth factors, human IL-3 is the most rapid evolutionary divergent. Our present data with homologous Rh-IL-3 convincingly demonstrate that the results of in vivo studies on human IL-3 in nonhuman primates have suffered from the considerable species specificity of IL-3 and are not representative of its full potential.

We predict that many of the effects of IL-3 can be selectively amplified if combined with lineage-specific growth factors, such as erythropoietin for RBCs and G-CSF, M-CSF,
or GM-CSF for cells of the granulocyte/monocyte series. Selective amplification of the response by other hematopoietic growth factors might well provide a most versatile use of IL-3 in medicine, and may reduce effective single therapeutic doses of IL-3 and lineage-specific hematopoietic growth factors to doses that more approach physiologic requirements, thus reducing side effects. The vast increase in intracellular peripheral blood histamine and stimulation of the production of eosinophilic granulocytes may demonstrate an important role of IL-3 in allergic and asthmatic reactions.

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IL-3 EFFECTS IN RHESUS MONKEYS

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