In Vivo Effects of Recombinant Human Interleukin-6 in Primates: Stimulated Production of Platelets

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In cynomolgus monkeys, twice daily subcutaneous injections of recombinant human interleukin-6 (rhIL-6) at doses of 5 to 80 µg/kg/d for 14 consecutive days caused dose-dependent increases in platelet count, usually continuing for more than 1 week after cessation of the injections. The count reached a level approximately twofold or more above the preinjection level even at 5 µg/kg/d, and at doses of more than 20 µg/kg/d, the increase became biphasic with a higher second peak 3 days after cessation of the injections. Morphologic analysis of the bone marrow after the 7 day-injections with 80 µg/kg/d revealed a marked increment in size of megakaryocytes compared with control, indicating the promotion of megakaryocyte maturation. Other changes attributable to the rhIL-6 treatment include dose-dependent loss of body weight, anemia, neutrophilia and monocytosis, elevation of serum C-reactive protein and alpha-1 acid glycoprotein levels, and decrease of serum albumin; all of which returned to normal within 1 week after cessation of the injections and were tolerable at doses of less than 10 µg/kg/d. These findings suggest that rhIL-6 may be an effective strategy for the treatment of thrombocytopenia.

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MATERIALS AND METHODS

Animals. Eight female adult cynomolgus monkeys (Macaca fascicularis) weighing 2.8 to 3.8 kg were kept individually in monkey stainless steel cages in an air-conditioned room at 25°C. They were bred under the same conditions throughout the experiments.

rhIL-6 and its administration. Recombinant human IL-6 was prepared by expressing a complementary DNA for human IL-6 in Escherichia coli followed by further purification, as previously described. The specific activity was 5 x 10⁴ U/mg protein, as estimated by using an Epstein-Barr virus-transformed SKW6-CL-4 cell line as a target. The rhIL-6 solutions for injections were freshly prepared by dissolving various amounts of rhIL-6 in sterile phosphate-buffered saline containing 1% heat-inactivated autologous monkey serum. As a control, a solution containing human serum albumin (HSA; Fuji Rebio Inc, Tokyo, Japan) instead of rhIL-6 was also prepared. Endotoxin contamination of these solutions was less than 0.03 ng/mg protein. Two milliliters of the solution containing 2.5, 5, 10, 20, or 40 µg rhIL-6 or 40 µg HSA were injected twice daily for 14 consecutive days (day 0 through Day 13) into the subcutaneous (SC) space of each monkey.

Hematologic examinations. Blood samples were drawn from the femoral vein of each monkey at various intervals. Complete blood cell counts were performed using a Sysmex automatical analyzer (Toa Medical Electrics Co, Hyogo, Japan) and differential white blood cell (WBC) counts were performed on smear preparations stained with May-Grunwald-Giemsa. Femur bone marrow samples were obtained at day 7 from one of the monkeys receiving 80 µg rhIL-6/kg/d and from one of the control monkeys. Differential cell counts and estimation of the size of megakaryocytes in marrow were performed on Cytospin preparations (Shandon Inc, Sewicke, PA).

Table 1. Increase in Platelet Counts in Peripheral Blood of Cynomolgus Monkeys in Response to rhIL-6 Treatments

<table>
<thead>
<tr>
<th>rhIL-6 (µg/kg/d)</th>
<th>Increase in Platelet Counts (x 10¹³/µL Blood)</th>
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<tbody>
<tr>
<td>0</td>
<td>Day 10</td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>25.2</td>
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<tr>
<td>10</td>
<td>16.2</td>
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<tr>
<td>20</td>
<td>5.7</td>
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<tr>
<td>40</td>
<td>15.0</td>
</tr>
<tr>
<td>60</td>
<td>26.6</td>
</tr>
</tbody>
</table>

Monkeys were treated twice daily SC with various doses of rhIL-6 over a period of 14 consecutive days (day 0 through day 13). Values indicate the increase in platelet counts over the pretreatment levels; maximal levels of the increase in platelet counts are underlined.

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IN VIVO EFFECTS OF IL-6

Changes in peripheral blood platelet counts in cynomolgus monkeys treated SC with rhIL-6 for 14 days (as indicated by arrows). Monkeys were treated twice daily with 5 to 80 μg/kg/d of rhIL-6. Control monkey received HSA.

stained with May-Grünwald-Giemsa and with a TP80 avidin-biotin kit using anti-human GPIIb/IIIa (CD41b) monoclonal antibody (Nichirei Co, Tokyo, Japan), respectively. Megakaryocyte size were expressed as the average of two perpendicular cell diameters of 100 CD41 b-positive cells. Using part of the femurs, histologic sections were also prepared by the conventional method and stained with periodic acid-Schiff (PAS).

Other examination. Sera separated from the blood samples obtained 1 day before the injections and 12 hours and 1 week after cessation of the injections were assayed for various parameters. Total proteins, albumin, glutamic oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), urea nitrogen (BUN), creatinine, total cholesterol (T-Cho), and triglycerides (TG) in sera were estimated using a 706D automatic analyzer (Hitachi Co, Tokyo, Japan), and alpha-1 acid glycoprotein (α1-AGP) and C-reactive protein (CRP) in sera by a conventional nephelometry assay.

RESULTS

Changes in blood cell counts. The rhIL-6 injections caused significant changes in blood cell counts in a dose-dependent fashion. The most remarkable change was a gradual increase of platelets, which began around day 7 and continued during the course of the injections (Fig 1). A transient fall soon after starting injections was observed in all the monkeys, including controls. Interestingly, at doses of more than 20 μg/kg/d the pattern was biphasic with peaks at day 7 and day 16, while at doses of 5 and 10 μg/kg/d, the
Changes in peripheral blood RBC counts in cynomolgus monkeys treated SC with rhIL-6 for 14 days. Monkeys were treated twice daily with 5 to 80 μg/kg/d rhIL-6. Control monkey received HSA. Data are expressed as a percentage of pretreatment levels. Pretreatment levels of RBC counts were 622, 664, 616, 688, 826, and 664 x 10^11/L blood in 0, 5, 10, 20, 40, and 80 μg/kg/d rhIL-6–treated monkeys, respectively.

Peak was observed at day 10 and day 14, respectively. Degree of the increase was two times or more above the preinjection levels at each dose, and the maximum increase in platelet counts obtained was proportionally related to the dose, as shown in Table 1. After cessation of the injections, the levels returned to normal, but the rate seemed to be slower at higher doses; at doses of more than 20 μg/kg/d, the counts at day 20 still exceeded the normal level.

In contrast, the moderate increase in neutrophils was observed at higher doses. The increase in monocytes and decrease in lymphocytes and red blood cells (RBCs) were also observed, but these levels returned to normal within 1 week after the cessation of injections at doses of less than 10 μg/kg/d (Figs 2 and 3). No changes in eosinophil and basophil counts were observed at any dose.

Bone marrow morphology. Differential counts of bone marrow cells at day 7 from the monkey receiving 80 μg/kg/d revealed relative myeloid hyperplasia (data not shown). Although there was no evidence of an increased megakaryocyte count in the rhIL-6–treated monkeys, the histologic section demonstrated the increment of megakaryocyte size by the rhIL-6 treatment, as shown in Fig 4. To confirm this observation, we compared the size distribution of megakaryocytes in the monkey receiving 80 μg/kg/d for 7 days and in the control monkey. As shown in Fig 5, the average megakaryocyte size from the rhIL-6–treated monkey was significantly larger than that from the control (33.3 ± 16.5 μm versus 24.9 ± 12.7 μm, P < .01), according to Student's t test.

Other changes. The loss of body weight was observed partly due to decreased diet intake. However, the weight loss was only of slight degree (5% to 8%) except for the monkey receiving the highest dose of rhIL-6 (80 μg/kg/d), and the weight returned soon after cessation of the injections. It was also found that various parameters in sera from the rhIL-6–treated monkeys became abnormal at day 14 in a dose-dependent fashion (Table 2). Of these abnormalities, the most striking were elevations of acute phase proteins, such as...
CRP and α1-AGP, associated with decreased albumin and T-Cho levels. However, these changes were also normalized within 1 week after cessation of the injections (data not shown). No significant changes of hepatic or renal function tests were observed.

DISCUSSION

As predicted from the murine work by Ishibashi et al., the rhIL-6 injections caused a dose-dependent increase of blood platelets in primates as well. It is quite interesting to note that the rhIL-6–induced increase of platelets was observed even at doses less than 10 μg/kg/d, doses that had only the minimum effects on the other parameters. Moreover, the pattern of the platelet increase was gradual or biphasic during the course of the rhIL-6 injections at higher doses of rhIL-6. The latter findings may indicate enhanced production of platelets by rhIL-6 administration. In fact, our study showed that the rhIL-6–induced increase of platelets was accompanied with the increment of larger megakaryocyte population, as observed in murine system. These findings support the view that IL-6 acts as one of the thrombopoietic factors in vivo.

An IL-6 transgenic mice study suggested that an increase in serum immunoglobulin and the development of plasmacytosis and mesangio-proliferative glomerulonephritis were observed only when a high level of serum IL-6 is maintained for a longer period. However, it is unlikely that such abnormalities are induced in monkeys treated with an appropriate amount of rhIL-6. Although further study will be needed to answer whether rhIL-6 might have any adverse effects of immunologic functions or whether the rhIL-6–induced platelets have normal functions, the present results in primate system strongly suggest that rhIL-6 may be valuable for the treatment of thrombocytopenia.

REFERENCES


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