Long-Term Interleukin-6 Administration Stimulates Sustained Thrombopoiesis and Acute-Phase Protein Synthesis in a Small Primate—The Marmoset


Interleukin-6 (IL-6) has been ascribed significant roles in both hematopoiesis and the immune response, although its contribution to host defense as a whole is poorly understood. Because short-term IL-6 treatment was previously shown to stimulate megakaryocyteopoiesis, we investigated the effect of long-term administration of IL-6 on megakaryocyteopoiesis and other systemic parameters in nonhuman primates. We chose a small primate, the marmoset (Callithrix jacchus), which enabled long-term administration at high doses. Recombinant human IL-6 (rhIL-6) administered at doses of up to 1,000 μg/kg/d over 4 and 9 weeks caused a sustained twofold to threefold increase of thrombocyte counts, peaking at 4 weeks. Thrombocyte counts declined thereafter, despite continuing IL-6 administration. The number of bone marrow megakaryocytes at 4 and 9 weeks was not increased compared with controls, but the ploidy grade was augmented, suggesting that IL-6 effects are restricted to mature megakaryocytes in vivo. An acute-phase protein response was observed within 24 hours after the first IL-6 administration and reached a maximum after 1 week of IL-6 administration at 25 μg/kg. Serum C-reactive protein, haptoglobin, and ceruloplasmin were increased, whereas albumin and transferrin levels declined. The acute-phase protein response was not associated with any morphologic evidence of hepatocellular damage. The increased levels of Ig and soluble IL-2 receptor in the serum levels reflected systemic immunostimulation. There was no evidence of renal mesangiolipropressive pathology. Antibodies against rhIL-6 developed within 2 weeks, continuously increasing during the course of the study. High titers of neutralizing antibodies appeared concomitantly with the decrease in platelet counts and decline in acute-phase proteins. Therefore, despite the pleiotropic effects of IL-6 observed in vitro, long-term administration of IL-6 caused a selective and sustained stimulation of thrombopoiesis in marmosets that was only ablated by the appearance of neutralizing antibodies, and high doses were well tolerated in marmosets. A long-term targeting of IL-6 to cells of the megakaryocytic lineage, without evoking general toxicity, confirms the potential therapeutic usefulness of rhIL-6 for the chronic treatment of thrombocytopenia patients.

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INTERLEUKIN-6 (IL-6) is a multifunctional cytokine produced by both lymphoid and nonlymphoid cell types. It has regulatory effects on the immune and hematopoietic systems, and causes the acute-phase reaction.\(^1\) Its pleiotropic nature is reflected by the various names originally given to this cytokine, including B-cell stimulation factor 2, plasmacytoma growth factor, hepatocyte stimulating factor, and interferon-β2. Molecular cloning showed that all these activities are encoded by the same gene, leading to a 21- to 28-kD glycoprotein.\(^3\) IL-6 exerts its biologic effects through a specific membrane receptor composed of two proteins, 80-kD and 130-kD (gp130) membrane proteins,\(^1\) the latter combining with the p80 receptor to initiate signal transduction.\(^8\) IL-6 receptors are expressed by many cell and tissue types, including megakaryocytes.\(^9\)

An accumulating body of evidence exists describing an important role for IL-6 in the early stages of hematopoiesis\(^10\)\(^11\) and later in differentiation and maturation of megakaryocytes.\(^2\) These hematopoietic activities are of special clinical interest, because they imply that IL-6 may be of therapeutic use in the treatment of several forms of thrombocytopenia. Investigations in healthy mice\(^13\)\(^14\) and primates\(^16\)\(^17\) showed that repeated daily injections of recombinant human IL-6 (rhIL-6) increased normal blood platelet counts by a factor of 2 to 3, associated with an increased megakaryocyte maturation. Furthermore, IL-6 was shown to enhance the recovery of megakaryopoiesis and thrombopoiesis after bone marrow damage.\(^20\)\(^21\)

Most of these studies investigating the effects of IL-6 on thrombopoiesis in rodents and primates have been short-term.\(^13\)\(^19\) We chose to investigate its long-term effects on animals using a protocol mimicking the chronic therapeutic application. Because of the high species-specificity of human-derived cytokines, a nonhuman primate, the marmoset (Callithrix jacchus), was selected. Their small size (approximately 350 g) allows long-term administration of high doses of recombinant material, rendering these infrequently used animals ideal in hematopoietic research. IL-6 showed potent sustainable thrombopoietic properties, with a peak after 4 weeks of daily administration. Thrombocyte counts decreased thereafter, concomitant with the occurrence of high titers of neutralizing anti-rhIL-6 antibodies. In view of the pleiotropic effects of IL-6, in particular the potentially adverse immunostimulatory,\(^13\) hepatic,\(^23\) and renal effects,\(^24\) it is important to establish a threshold for pharmacologic effects and define a therapeutic window. Our investigations demonstrate the potential usefulness of long-term therapeutic application of IL-6, with high specificity for the induction of thrombocytopenia, in the absence of significant adverse effects.

MATERIALS AND METHODS

Experimental design. Adult male and female marmosets (Callithrix jacchus) were housed individually in air-conditioned rooms maintained at 25°C and 60% humidity. The animals had free access to food (lab chow and fresh fruit) and water. Experimental groups consisted of 3 male and 3 female animals.

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Administration. rhIL-6 (Escherichia coli derived) was a generous gift from Sandoz Pharma (Basel, Switzerland). rhIL-6 was dissolved at 1 mg/mL and diluted in saline containing 1% marmoset serum. Single daily subcutaneous injections (1 mL/kg) of 0, 25, 100, and 500 µg/kg were administered for 9 weeks, or at 5 and 1,000 µg/kg for 4 weeks. A third group injected at daily doses of 500 µg/kg for 4 weeks were analyzed after a 4-week recovery period.

Hematology and clinical chemistry. Blood was drawn from the femoral vein of each monkey at 0, 1, 2, 4, 7, and 9 weeks (24 hours postinjection) and after a 4-week recovery period. Complete blood counts were performed with a Sysmex E2500 hematology analyzer (Digitana, Switzerland) and differential white blood cell (WBC) counts were performed on smear preparations stained with May-Grünwald Giemsa. Sera separated from the blood samples were assayed for renal and hepatic functional parameters on a Cobas Fara chemistry analyzer (Roche, Basel, Switzerland; creatinine, urea, liver enzymes, bilirubin, protein, albumin). Serum electrophoresis (Beckman, Nyon, France) was performed routinely and analyzed on a Masterscan densitometer (Scanalytics; CSPI, Billerica, MA). Soluble IL-2 receptors in sera were measured by enzyme-linked immunosorbent assay (ELISA; Bühlmann, Basel, Switzerland).

Bone marrow cell analysis. One milliliter of bone marrow was obtained from control and IL-6–treated animals at 1 and 2 weeks, and layered on a discontinuous isotonic Percoll gradient (Pharmacia, Uppsala, Sweden) at a density of 1.058 g/mL. Cells were collected from the interface and the top of the gradient as described. DNA was labeled using propidium iodide (50 µg/mL; Sigma, St Louis, MO). Labeled cells (2 × 106) were analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA). DNA ploidy distributions were analyzed from megakaryocytes ≥8 N as described.

Acute-phase proteins. C-reactive protein (CRP), ceruloplasmin (CER), haptoglobin (HPT), and transferrin (TFR) were determined in serum by rocket immunoelectrophoresis as described. Antibodies were obtained from Dako (Glostrup, Denmark).

Serum rhIL-6 levels. rhIL-6 levels were determined using a two-sided sandwich immunosassay. Flexible microtiter plates were coated with 7.5 µg/mL mouse-α–rhIL-6 antibodies (Sandoz Pharma). After blocking the plates with 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), rhIL-6 standards and test samples in 1% BSA/PBS were incubated for 1 hour at 37°C followed by rabbit-α–rhIL-6 (Ryffel, 1:10,000). The plates were washed and the complex was developed with goat-α-rabbit antiserum phospho-

Table 1. Hematologic Effect of rhIL-6 in Marmosets

<table>
<thead>
<tr>
<th>IL-6 Dose* (µg/kg/d)</th>
<th>WBC (10⁹/µL)</th>
<th>RBC (10⁹/µL)</th>
<th>Thrombocytes (10⁹/µL)</th>
<th>M/E Ratio</th>
<th>Megakaryocyte No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.300 ± 1.333</td>
<td>6.68 ± 0.39</td>
<td>567 ± 203</td>
<td>2.2</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>25</td>
<td>9.067 ± 2.996</td>
<td>8.88 ± 2.81</td>
<td>1.399 ± 2154</td>
<td>2.0</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>100</td>
<td>11.217 ± 5.081</td>
<td>9.52 ± 3.26</td>
<td>1.364 ± 4744</td>
<td>2.1</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>500</td>
<td>12.983 ± 1.411†</td>
<td>9.23 ± 2.25</td>
<td>1.523 ± 4144</td>
<td>1.9</td>
<td>14 ± 4</td>
</tr>
</tbody>
</table>

* rhIL-6 was administered SC at daily doses, with examinations at 2 weeks.
† Bone marrow was analyzed at 9 weeks: M/E, myeloid/erythroid precursor ratio; megakaryocyte number per low power microscopic field.
‡ Statistical difference from controls, P < .05 (Student's t-test).
The effect of rhlL-6 administration (1,000 μg/kg/d) for 4 weeks on the bone marrow of control (A) and IL-6 (1,000 μg/kg) -treated monkeys (B). Hemalaun and eosin, original magnification ×200. (C) Size distribution of bone marrow megakaryocytes from (■) control and (□) IL-6–treated (500 μg/kg) marmosets, as described in the Material and Methods section. (D) Serial-gated DNA histograms of bone marrow cells by flow cytometry at 1 week as described in the Materials and Methods. Baseline histogram was obtained from control marmosets (a). The modal ploidy was 32 N at 5 μg/kg (b) and reached 64 N at 25 μg/kg (c) and 100 μg/kg (d).

Table 2. rhlL-6 Increases Soluble IL-2 Receptors in Serum and Tissue

<table>
<thead>
<tr>
<th>IL-6 Dose (μg/kg/d)</th>
<th>1 wk</th>
<th>2 wk</th>
<th>4 wk</th>
<th>9 wk</th>
<th>CD25 and Class II Expression†</th>
<th>Follicle Size† (relative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>42 ± 6</td>
<td>27 ± 7</td>
<td>32 ± 8</td>
<td>46 ± 4</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>25</td>
<td>151 ± 44</td>
<td>214 ± 14</td>
<td>202 ± 18</td>
<td>78 ± 41</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>100</td>
<td>118 ± 22</td>
<td>75 ± 31</td>
<td>182 ± 24</td>
<td>38 ± 7</td>
<td>2.1</td>
<td>2.4</td>
</tr>
<tr>
<td>500</td>
<td>116 ± 7</td>
<td>245 ± 89</td>
<td>194 ± 34</td>
<td>13 ± 4</td>
<td>2.4</td>
<td>2.9</td>
</tr>
</tbody>
</table>

* Determined by ELISA; control values are in the 30 to 50 IU range.
† Semiquantitative score of CD25 and class II antigen expression (staining of frozen sections by APAAP technique) in lymphoid cells form the spleen at 9 weeks (0 to 5+).
‡ Relative size of B-cell area in the spleen compared with controls as described in Materials and Methods.
Fig 3. Serum electrophoretograms from control (A) and IL-6 (1,000 µg/kg)–treated marmosets at 3 (B) and 14 days (C). These electrophoretograms are representative for six animals in each group.

B13 bioassay. The neutralizing property of the antibodies was determined by assessing the IL-6–dependent proliferation of the B13 subclone of a murine hybridoma as described.

Histology. The animals were killed by halothane narcosis. The organs were weighed and fixed in 4% buffered formalin, paraffin embedded, cut at 5 µm, and stained by hematoxylin-eosin and chromotrope aniline blue.

Frozen tissues from liver, kidney, spleen, thymus, and lymph nodes were prepared with a cryomicrotome, fixed in acetone for 10 minutes, and stored at −70°C until use. Incubations were performed with CD25, CD3, IgG, L26 (B-cell) antibodies and anti-class II histocompatibility antigen antibodies (HLA-DR; Dako) and NKH-1(NK) (Coulter, Zurich, Switzerland). The first antibody was developed with rabbit-antimouse Ig coupled to alkaline phosphatase.

Proliferation of mesangial cells was assessed by incubation of formaldehyde-fixed renal sections with murine antihuman PCNA antibody (Dako) followed by the ABC elite detection system according to the manufacturer’s instructions (Vector Lab, Burlingame, CA). The presence of PCNA-labeled mesangial cells was counted for 50 glomeruli per animal.

Electronmicroscopy. Liver and kidney tissue were fixed in 3% glutaraldehyde, postfixed in osmium, and Epon embedded, and ultrathin sections were examined with a Philips electron microscope (Philips Ltd., Holland).

Morphometric analysis. Bone marrow sections were obtained from the femur; the tissue was decalcified, cut at 3 µm, and stained with Giemsa. The megakaryocyte size was estimated by the average of two perpendicular cell diameters of 100 megakaryocytes in the

Table 3. Effects of rhIL-6 on Acute-Phase Proteins in Serum of Marmosets

<table>
<thead>
<tr>
<th>IL-6 Dose (µg/kg/d)</th>
<th>HPT (mg/mL)</th>
<th>CRP (mg/mL)</th>
<th>CER (mg/mL)</th>
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<tbody>
<tr>
<td></td>
<td>1 wk</td>
<td>2 wk</td>
<td>4 wk</td>
</tr>
<tr>
<td>0</td>
<td>1.3</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>7.1</td>
<td>9.4</td>
<td>8.2</td>
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<tr>
<td>25</td>
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<td>16.4</td>
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<td>15.5</td>
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<td>14.7</td>
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<tr>
<td>500</td>
<td>16.1</td>
<td>22.1</td>
<td>11.6</td>
</tr>
<tr>
<td>1,000</td>
<td>17.6</td>
<td>21.4</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Table 4. Renal and Hepatic Effects of rhIL-6 in Marmosets

<table>
<thead>
<tr>
<th>IL-6 Dose (µg/kg)</th>
<th>Urea (mg/100 mL)</th>
<th>Creatinine (mg/100 mL)</th>
<th>GOT (IU/L)</th>
<th>GPT (IU/L)</th>
<th>Protein (g/L)</th>
<th>Albumin (g/L)</th>
<th>Total Ig (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15.3</td>
<td>1.1</td>
<td>34</td>
<td>26</td>
<td>7.2</td>
<td>3.8</td>
<td>1.8</td>
</tr>
<tr>
<td>25</td>
<td>16.9</td>
<td>1.0</td>
<td>27</td>
<td>31</td>
<td>6.8</td>
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<td>2.1</td>
</tr>
<tr>
<td>100</td>
<td>14.8</td>
<td>0.9</td>
<td>14</td>
<td>24</td>
<td>6.7</td>
<td>3.1</td>
<td>2.3</td>
</tr>
<tr>
<td>500</td>
<td>16.4</td>
<td>1.1</td>
<td>25</td>
<td>28</td>
<td>6.5</td>
<td>2.7</td>
<td>2.6</td>
</tr>
</tbody>
</table>

* rhIL-6 was administered SC at daily doses, with examination at 4 weeks.
bone marrow section. The relative size of various areas in the lymph node, e.g., lymph follicle, paracortex, and medulla, was assessed planimetrically from cervical lymph nodes as described previously. The relative size of red and white pulp in the spleen and of cortex in medulla of the thymus were assessed by the same method.

RESULTS

**IL-6 stimulates thrombopoiesis.** rhIL-6 was administered daily for 4 and 9 weeks by subcutaneous (SC) injection. IL-6 was well tolerated at the highest dose of 1,000 µg/kg SC. There were no severe clinical side effects, rash, fever, or decrease in body weight. The absence of any significant increases in the body temperature was surprising; however, there are quite large diurnal variations in marmosets. The cutaneous injection site was unremarkable. IL-6 caused a twofold to threefold increase in thrombocyte counts (Fig 1). Even at the low dose of 25 µg/kg, a more than twofold increase of thrombocyte counts was observed within 2 weeks. The effect was sustained and maximum thrombocyte counts were reached at 4 weeks. Thrombocyte counts decreased thereafter, despite continued injection of rhIL-6. This decrease is unlikely caused by a resistance or exhaustion, but is rather caused by the formation of neutralizing antibody to rhIL-6 (see below). The IL-6 effect was quite selective on thrombopoiesis; however, a small, significant (500 µg/kg dose) dose-dependent augmentation of WBC counts occurred within 1 to 2 weeks that subsided after 3 to 4 weeks (Table 1). Red blood cell (RBC) numbers were slightly increased.

Bone marrow cellularity appeared normal at 9 weeks, with all elements of the erythroid and myeloid series present in normal proportions. The megakaryocyte count did not differ in IL-6-treated versus control animals (Table 1). However, the average size of the megakaryocytes was increased by IL-6 treatment on histologic sections (Fig 2A and B). The mean diameter as a measure of size was distinctly augmented (Fig 2C). An examination of bone marrow at 1 and 4 weeks gave qualitatively the same results. A ploidy analysis at 1 week showed a significant shift with a large increase in the frequency of 64 and 128 N megakaryocytes (not shown).
Serial DNA histograms showed a large increase of the normal 16 N modal ploidy to a modal ploidy of 64 N within 1 week of IL-6 administration (Fig 2D). The shift of the modal ploidy was already observed at the 5 μg/kg doses and was maximal at 25 μg/kg. The platelet volumes were in the normal range and the morphology assessed on the cytologic preparations was normal. Functional studies have not been performed. The reversibility of the thrombopoietic effects of IL-6 was assessed after 4 weeks of treatment followed with a 4-week period with no injections. The platelets counts decreased to the normal range within 2 weeks upon cessation of IL-6 administration and the ploidy of bone marrow megakaryocytes was normalized at 4 weeks (data not shown). The circulating levels of soluble IL-2 receptor were raised at 1 week and continued to increase till 4 weeks, declining thereafter (Table 2). Expression of the IL-2 receptor (CD25), MHC class II antigen (Table 2), and NK markers were increased in the lymphoid system (R. Neubert, manuscript in preparation). Whereas the general architecture of lymphoid tissue was normal and spleen and thymus weights were unchanged, morphometric analyses of cortex, paracortex, and medulla of cervical lymph nodes showed a distinct increase in the size of the cortical follicles in the IL-6-treated animals (Table 2). The splenic follicles appeared also slightly increased, but no alterations were observed in the thymus. Thus, an unequivocal stimulatory effect of IL-6 on the immune system occurred without adverse side effects.

Hepatic acute-phase response. The composition of serum proteins showed marked changes after IL-6 administration. Figure 3A shows a representative electrophoretogram of a control animal. In marmosets treated at 1,000 μg/kg for
IL-6 IN MAMMALS

Fig 7. Electron microscopy of renal glomeruli from a monkey treated with 1,000 µg/kg rIL-6 for 4 weeks. Original magnification x 18,000.

3 days (Fig 3B) and for 14 days (Fig 3C), acute-phase protein peaks were observed in the α, β1, β2, and β3 globulin fractions, with γ globulins being elevated only after 2 weeks. Reduction of albumin was observed in treated animals. The synthesis of the typical acute-phase proteins, namely HPT, CER, and CRP, was confirmed by rocket immunoelectrophoresis (Table 3). A significant increase of HPT was detectable after the administration of 5 µg/kg rIL-6 and was almost maximal at the 25 µg/kg dose (Fig 4A). HPT levels were elevated during 1 and 2 weeks, whereas they were significantly reduced at 4, 7, and 9 weeks, possibly because of the formation of neutralizing antibodies that may also have inhibited endogenous IL-6 (Fig 4B). Ceruloplasmin levels increased within 8 to 24 hours and decreased after 4 weeks of IL-6 treatment (Fig 4C). Hepatic stimulation by rIL-6 was not associated with the release of transaminases or alkaline phosphatase usually indicative of liver damage (Table 4). Livers were not enlarged, and no degenerative or hypertrophic hepatocellular changes were observed by light microscopy. The Kupffer and endothelial cells appeared activated after 1,000 µg/kg IL-6, and the sinusoids were populated by mononuclear cells (Fig 5). Immunohistochemistry showed L26-positive B cells, activated T cells (CD25+), and abundant NK cells (not shown). An increase of NK cells was also found in the circulation within 2 weeks of IL-6 administration (D. Neubert, manuscript in preparation). IL-6 was therefore able to induce an immediate acute-phase serum response not associated with hepatic parenchymal alteration.

Renal function and morphology. Because IL-6 induced mesangioproliferative glomerulonephritis in IL-6 transgenic mice,15 we investigated renal function and morphology in detail. Renal function was normal as assessed by serum levels of creatinine and urea and by the absence of proteinuria or microhematuria. Even at the highest dose of IL-6, the glomeruli appeared normal with a slender mesangium (Fig 6). A few mononuclear cells were found in the periglomerular interstitial space. The proliferative status of mesangium cells was assessed by the use of the proliferation marker PCNA.24 The percentage of PCNA-positive mesangial cell nuclei was not increased by IL-6 (data not shown). Electron-microscopy showed minimal electron-dense deposits in the glomerular basement membrane without any inflammatory cell reaction or any mesangioproliferative alterations in the glomerulus (Fig 7). Renal function and morphology were therefore unaffected by long-term and high-dose IL-6 treatment in marmosets.

Bioavailability and immunogenicity. rIL-6 was measured in the serum by ELISA. Peak levels were reached between 1 and 2 hours after a single-dose SC injection of 100 µg/kg or 1,000 µg/kg, and were in the range of 7 to 10 ng/mL and 75 ng/mL, respectively (Fig 8). The half-life (T½) for IL-6 was estimated as 4 to 6 hours (Table 5). The AUC (area under the concentration-time curve) increased about 10-fold, indicating a linear absorption of IL-6. Antibodies directed against rIL-6 appeared after 2 weeks and increased in titers up to 9 weeks (Table 6). Every single animal developed antibodies against rIL-6. These antibodies neutralized the IL-6 effects as measured by the B13 bioassay. Their neutralizing property in vitro became apparent between 4 and 6 weeks (not shown). The bioavailability after repeated injections was distinctly reduced in hyperimmune animals (data not shown). Thus, rIL-6 was found to be immunogenic in the marmoset, as in other nonhuman primates.18 This limits long-term studies to 2 to 6 weeks and thus hampers chronic investigations.

DISCUSSION

The potent thrombocytogenic effect of IL-6 has been established for both murine and primate species.13-14 Investigations in healthy mice15 and primates16 demonstrated that repeated daily injections of IL-6 increased normal blood platelet counts twofold, which is associated with signs of increased megakaryocyte maturation. Furthermore, IL-6 was shown to enhance the recovery of megakaryocyte and thrombopoiesis after bone marrow damage.20-22 Most studies investigating IL-6 effects on thrombopoiesis, however, have been short-term.11,12 IL-6 was administered at 30 µg/kg/d SC for 8 days in rhesus17 and up to 80 µg/kg for 14 days in cynomolgous monkeys.18 Prolonged stimulation of the hematopoietic system by
growth factors may potentially evoke secondary bone marrow failure through exhaustion of precursor cells after an exaggerated turnover of these cells. The recombinant protein itself may also be recognized by the immune system, with resulting antibodies potentially neutralizing endogenous growth factors and suppressing normal hematopoiesis; this latter event is more likely to occur in animals treated with the recombinant human-derived proteins.

We therefore chose to examine the effect of long-term IL-6 therapy on thrombogenesis in primates. Using a small primate, the marmoset, we confirmed a time- and dose-dependent stimulation of thrombopoiesis. This was reflected by a steady increase in platelets counts up to 4 weeks. This effect on thrombopoiesis was fully reversible within 2 weeks upon cessation of IL-6 administration. The modal ploidy of bone marrow megakaryocytes was normalized at 4 weeks. Thrombocyte morphology and volume were unchanged. The resistance to L-6 developing during long-term administration was most likely caused by the formation of neutralizing antibodies to the human protein, which developed between 4 and 6 weeks. Bone marrow megakaryocytes showed distinct sustained volume and ploidy increases. The earliest changes were observed after 1 week. Stahl et al reported a significant increase of megakaryocyte ploidy within 3 days in rhesus monkeys. Significantly, in our study, no signs of exhaustion of megakaryopoiesis were observed after 9 weeks of IL-6 administration. Despite the occurrence of neutralizing antibodies, there was no sign of bone marrow damage, a potentially serious adverse effect should antibodies neutralize endogenous host IL-6. Therefore, the effect on megakaryocyte differentiation, maturation, and release of mature platelets by IL-6 was quite selective, because most other hematologic parameters were essentially unchanged.

In view of the pleiotropic range of activities demonstrated for IL-6 in vitro, systemic effects of IL-6 with respect to the immune system, liver, and kidney were examined. The effects on the immune system were significant, although unassociated with signs of toxicity such as the vascular leak syndrome. Immunostimulation by IL-6 was confirmed by increased serum Ig and soluble IL-2 receptor levels in the serum. Moreover, B-cell areas, including the lymphoid folli-
cles of the spleen and lymph nodes, were enlarged, expressing increased numbers of activated T lymphocytes and NK cells. This immunostimulatory property has been described previously33,35 and is considered central to the experimentally observed antitumor effect of IL-6 in mice.36 The induction of NK cells in vivo, however, has not been described.37 Immunostimulation was not accompanied by the induction of autoimmune disease in the marmosets.

IL-6 caused marked synthesis and release of acute-phase proteins in the serum,23,33 but without accompanying systemic signs of an acute-phase response such as fever or loss of body weight. The absence of any systemic effect, including fever or skin erythema, is in contradistinction with the early clinical experience with IL-6 in cancer patients.42

IL-6 has been shown to enhance the growth of renal mesangial cells and to play a central role in the development of mesangio-proliferative glomerulonephritis.34,38 Morphologic investigations of the kidneys by electron microscopy and immunohistochemistry did not show glomerular pathology. The presence of IL-6 in the mesangium, as reported in patients with mesangio-proliferative glomerulonephritis,34,38 appears insufficient to initiate mesangial pathologic alterations.

Antibodies to rhIL-6 developed within 2 weeks and increased up to 4 and reached a maximum at 9 weeks. As expected rhIL-6 was immunogenic in every single animal. IL-6 itself may enhance this antibody formation through its immunostimulatory actions. After SC administration, rhIL-6 levels were readily detectable in the serum within a few hours. The occurrence of neutralizing antibodies within 4 to 6 weeks, as assessed by bioassay, decreased the bioavailability of rhIL-6 and its biologic effect, as previously described in rodents.36

Human IL-6 caused a marked, long-lasting enhancement of thrombopoiesis in normal marmosets that, after 4 weeks, was blunted by the appearance of neutralizing antibodies. Exhaustion of precursor cells did not occur, even after 9 weeks of administration, as normal megakaryocyte counts were recorded at this time. IL-6 was well tolerated and signs of organ-specific and/or systemic toxicity were not observed. Other cytokines demonstrated to potentiate thrombopoiesis in vivo include leukemia inhibitory factor (LIF) in normal, 5-fluorouracil (5FU)-treated, and irradiated mice39 and rhesus35, IL-3 in patients with aplastic anemia35, and stem cell factor (SCF, Kit-ligand) in 5FU-treated mice.40 The targeting of LIF, IL-3, and SCF appears directed to immature precursors of megakaryocytes, whereas the effect of IL-6 in vivo is apparently restricted to more mature megakaryocyte precursors and megakaryocytes, given the observation that only marginal acceleration of platelet generation occurs in response to IL-6 in 5FU-treated mice41 and that megakaryocyte numbers were not elevated in the present study.

That IL-6 was well tolerated and efficacious in the absence of organ-specific and/or systemic toxicities in this long-term study points to a promising role for IL-6 in the treatment of thrombocytopenic patients when an adequate pool of mature megakaryocytes exists. In addition, in view of its main activity on late megakaryocyte precursors, the therapeutic effect of IL-6 might be optimal in combination with cytokines influencing earlier stages of megakaryopoiesis. Preliminary data from patients treated with rhIL-6 confirm the potent thrombopoietic effect, but also show unpredicted adverse effects such as fever, erythema, and mild increments of hepatic enzymes.45 Such discrepancies are not unknown in medical toxicology and demonstrate again that experimental animal data may be useful but can not be directly extrapolated to the human situation.

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Long-term interleukin-6 administration stimulates sustained thrombopoiesis and acute-phase protein synthesis in a small primate--the marmoset