Recombinant Human Leukemia Inhibitory Factor Induces Acute Phase Proteins and Raises the Blood Platelet Counts in Nonhuman Primates


Recombinant human leukemia inhibitory factor (rhLIF) produced by Escherichia coli was administered subcutaneously (sc) to rhesus monkeys at doses of 2, 10, and 50 µg/kg body weight/d for 14 days to assess its biologic activities in vivo. Serum levels of positively regulated acute phase proteins (APP) (C-reactive protein, α1-antitrypsin, haptoglobin, and ceruloplasmin) were increased, whereas the negatively regulated APP prealbumin decreased in response to rhLIF treatment. During the second week of treatment, blood platelet counts began to increase, resulting in a maximum of a twofold increase above normal levels a week after termination of the rhLIF treatment. No changes were seen in total and differential white blood cell counts in blood progenitor levels and in red blood cell numbers. The low- and medium-dose rhLIF treatments were tolerated without significant side effects. The animals treated with the high dose showed a reduction in body weight of approximately 10%. In conclusion, rhLIF was shown to stimulate APP and to increase the number of platelets in circulation in nonhuman primates.© 1993 by The American Society of Hematology.

Materials and Methods

Eight adult (four male, four female) Rhesus monkeys, Macaca mulatta, approximately 8 to 15 years old and weighing between 8 and 11 kg, were housed individually and provided with 10 changes per hour of 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 were provided with tap water ad libitum and commercial primate chow and fruit. Research was conducted according to the principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, USA.

Sources of rhLIF

The rhLIF used in this study was provided by the Sandoz AG, Basel, Switzerland. The nonglycosylated protein was extracted from E. coli cells expressing the LIF cDNA from a plasmid vector. The rhLIF that accumulated intracellularly was purified to homogeneity by a series of chromatographic steps, including high performance liquid chromatography (HPLC). The in vitro biologic activity of the highly purified rhLIF analyzed in a proliferation assay of the DA1 murine leukemic cell line was found to be 8.7 × 10^8 U/mg protein. The units were calculated on the basis that 1 U of rhLIF was able to induce 50% of maximal proliferation. The endotoxin content was 87 to 175 pg/mg as determined by the Limulus assay (Limulus amoebocyte lysate assay, Whitacker Bioproducts, Walkersville, MD).

Administration of rhLIF to Monkeys

The rhLIF was prepared for the subcutaneous (sc) treatment by thawing the daily dose and diluting it with 4 mL of nonpyrogenic saline containing 0.5% monkey serum. Two milliliters of the rhLIF solution was administered sc twice a day (at 9 AM and 6 PM) over a period of 14 days. The vehicle-treated control monkeys received sc injections of nonpyrogenic saline, supplemented with 0.5% monkey serum containing the same amount of endotoxin (lipopolysaccharide [LPS]; WHO standard from E. coli) as in the high dose of the rhLIF preparation.

Hematologic Examinations

PB for hematologic examinations was collected in EDTA-coated tubes before treatment, daily or at 2-day intervals during the treatment period, and three times a week during the posttreatment period.

Parameters measured included the total counts of red blood cells (RBCs), white blood cells (WBCs), platelets, and determination of the amount of hemoglobin and hematocrit (Sysmex 2000; TOA,
Medical Electronics, Kobe, Japan). Differential blood cell counts were established as normal for Rhesus monkeys on the examination of 200 cells of Giemsa-stained blood smears by two independent observers.

Hematologic examinations were expanded to include the determination of T and B cells by indirect immunofluorescence staining. Briefly, buffy-coat leukocytes freed of RBCs by hypotonic shock were incubated in microtiter plates with monoclonal antibodies (MoAbs), directed against cell surface structures on human lymphoid cells, which included CD2, CD3, CD4, and CD8 (Becton Dickinson, Mountain View, CA). After marking the bound MoAbs with fluorescinated antimouse Ig, the cells were analyzed by flow cytometry in a Coulter EPICS-753 flow cytometer (Coulter, Hialeah, FL).

Myeloid (colony-forming unit granulocyte-macrophage [CFU-GM]), erythroid (burst-forming unit-erythroid [BFU-E]), multipotential (CFU-mix), and megakaryocyte (CFU-Mk) progenitor cell numbers were determined by clonal assays, as previously described. Briefly, 2 × 10⁵ peripheral blood mononuclear cells (PBMC) harvested after Ficoll gradient centrifugation (Pharmacia, Uppsala, Sweden) were cultured in Iccove’s modified Dulbecco’s medium (IMDM) (GIBCO, Paisley, Scotland) with 0.8% methylcellulose, 30% fetal calf serum (FCS), and 10% bovine serum albumin (BSA). Clonal growth was stimulated by adding 5% phytohemagglutinin (PHA)-stimulated human leukocyte-conditioned medium, and 1 U/mL erythropoietin (Epo) (Toyobo, Osaka, Japan). After 14 days (37°C, 5% CO₂, full humidity), the cultures were classified microscopically. The colony numbers were expressed per 1 mL of blood.

Bone Marrow (BM) Biopsies

BM biopsies were taken from the ileal crest of anesthetized monkeys (10 mg Ketalar/kg [Parke Davis, Berlin, Germany], and 5 mg/kg Rompun [Bayer Ag, Leverkusen, Germany]) by a Yamshidi bone biopsy needle (Pharmaseal Lab, Glendale, CA) before and 1 day after high-dose (50 pg/kg/d) rhLIF treatment. The formalin-fixed decalcified BM cylinders were cut in 5-µm slices and stained with hematoxylin-eosin.

Determination of Serum Proteins of the Acute Phase Response (APR)

Serum C-reactive protein (CRP), α₁-antitrypsin, haptoglobin, and prealbumin were assayed by a nephelometric method using rabbit antisera against human proteins. The immune complexes formed were determined nephelometrically (Behring Nephelometer; Behring, Marburg, Germany). The amount of serum proteins was read from a standard curve established by various dilutions of a standard human serum, and were expressed as micrograms per milliliter of serum. Serum ceruloplasmin levels were determined by a radial immunodiffusion assay in agar containing rabbit antihuman ceruloplasmin antiserum, and using human ceruloplasmin serum as standard (I.C Partigen Behring, Marburg, Germany).

Determination of Body Weight, Behavioral Changes, and Food Intake

Each monkey was weighed before treatment, three times a week during the treatment period, and 2 to 3 weeks after termination of the rhLIF administration. Behavioral changes and food intake were observed on a daily basis.

Clinical Chemistry

Serum glutamic-pyruvic transaminase (SGPT) was assayed in monkey serum using a UV-method with nicotin amid-adenin-dinucleotid (NADH; Boehringer Mannheim [BM], Germany; BM Test-kit No. 258822). Cholesterol and triglycerides were determined by an enzymatic test (BM Testkit No. 701882). Creatinine was assayed with picric acid (BM Testkit Nos. 124192 and 290319). Total protein was determined by a modified biuret method. Albumin was measured with bromocresol green. The amounts of serum sodium, potassium, and calcium were determined using a flame photo-meter (Eppendorf, Hamburg, Germany). Chloride was measured with chloranilate of mercury. Magnesium was determined by a colorimetric assay using Merck Testkit No. 3338-0001 (Darmstadt, Germany).

Assaying Interleukin-6 (IL-6) in Blood Plasma of rhLIF-Treated Monkeys

Monkey IL-6 was determined in blood plasma samples by an immunoassay developed for assaying human IL-6 (Quantikine R & D Systems Inc, Minneapolis, MN). Blood was collected from high-dose-treated rhLIF monkeys in EDTA-coated tubes at 0, 1, 2, 3, 4, 6, 8, and 24 hours after the sc administration of rhLIF in the morning on day 7 of the treatment period. In addition, 50 µg rhLIF/kg was administered as a single intravenous (IV) bolus injection to an additional two monkeys. As a positive control, 100 ng endotoxin/kg (Salmonella abortus equi; Difco, Detroit, MI) was administered as IV bolus injection to two monkeys. Blood was collected at time points indicated above.

Assaying rhLIF Antibodies in the Sera of Monkeys Treated with rhLIF

Sera were analyzed for the presence of rhLIF antibodies by an enzyme-linked immunosorbent assay (ELISA) as previously described. Briefly, micro ELISA plates (Nunc, Roskilde, Denmark) were coated with rhLIF (10 µg/rhLIF/mL bicarbonate buffer pH 9.6 at 37°C for 60 minutes). A standard solution of Rhesus monkey IgG (Sigma, Deissenhofen, Germany) was also set up in a serial dilution starting at a concentration of 1 µg/mL. After repeated washings, serial twofold dilutions of test sera were added to the wells for 60 minutes at 37°C. After several washing steps, anti-monkey Ig enzyme conjugate (Nordic Immunology, Tilburg, The Netherlands) was added and incubated at the same conditions as mentioned above. The colorimetric reaction for detecting the bound antibodies was started by adding the enzyme substrate (Orthophenyl-Diamin [BioRad, Dusseldorf, Germany]) + 3% H₂O₂, after stopping the reaction by adding 100 µL 4 mol/L H₂SO₄, absorbance at 492 nm was measured in a microtiter spectrophotometer (Labinstruments, Salzburg, Austria), and the optical densities were related to the concentration of purified monkey IgG standard preparation and expressed as µg of anti-rhLIF IgG/mL serum.

The ability of anti-rhLIF antibodies to neutralize the in vitro biologic effect of rhLIF was determined by incubating 0.6 ng/mL rhLIF with fourfold dilutions (ranging from 1:4 to 1:1024) of monkey sera collected from the animals receiving the high-dose rhLIF treatment and showing high antibody titers in the ELISA system. Sera from these animals collected before treatment served as the control. The biologic activity of rhLIF was assayed by suppressing the growth of the M1 leukemic cell line.

RESULTS

Elevation of Blood Platelet Counts in Monkeys in Response to rhLIF Treatment

As shown in Fig 1, monkeys responded to the rhLIF treatment with a maximum twofold increase in blood platelet counts. The animals that received 2 µg rhLIF/kg/d showed an approximately 1.3-fold increase in maximum platelet counts, compared with pretreatment levels. An approxi-
approximately 1.4-fold increase of maximum platelet counts was achieved in monkeys treated with 10 μg rhLIF/kg/d. Platelet counts became elevated about twofold above pretreatment levels when the animals were treated with 50 μg rhLIF/kg/d. In animals treated with the lower doses of 2 and 10 μg rhLIF/kg/d, platelet counts began to increase during the last 2 days of rhLIF treatment, and reached maximum levels by day 8 after termination of the rhLIF administration. In monkeys treated with the high dose of 50 μg rhLIF/kg/d, platelet counts were above pretreatment levels by day 7 after initiation of the rhLIF administration, and increased steadily to maximum levels by day 4 after termination of treatment. Thereafter, platelet counts began to decrease, reaching normal levels during the end of the second week after termination of treatment.

**Effect of rhLIF Treatment on WBC and RBC Counts**

Treatment with a daily dose of 2, 10, or 50 μg rhLIF/kg did not induce significant changes in WBC counts. Lymphocyte subsets were determined by the expression of different cell-surface markers before, during, and after rhLIF treatment. No major changes in the total number of T cells (CD2+), the ratio of T-helper cells (CD4+) to T-suppressor cells (CD8+), and in the B cell population (CD20) were observed during the treatment and posttreatment observation periods (results not shown). No changes were found in RBC, reticulocyte counts, hematocrit, and hemoglobin levels either during or after rhLIF treatment. Also, no significant changes were seen in the progenitor cell numbers of the various lineages in the circulation of the monkeys throughout the rhLIF treatment and posttreatment periods, compared with pretreatment values (results not shown).

**Effect of rhLIF Treatment on BM Cellularity and Megakaryocyte Numbers**

BM cellularity was not changed significantly in the BM biopsies obtained before and after rhLIF treatment. Also, no significant changes in the number and size of megakaryocytes were observed in BM biopsies taken before and after high-dose rhLIF treatment.

**rhLIF Induced Changes in the Levels of APP**

**Elevation of CRP levels by rhLIF.** Figure 2 shows the increase in the mean CRP serum levels of the three monkey groups in response to the administration of 2, 10, and 50 μg rhLIF/kg/d. In the two animals receiving vehicle treatment serum, CRP levels remained below the detection limit of the assay (<3 μg CRP/mL). A daily dose of 2 μg rhLIF/kg increased CRP levels to a maximum of 2.4-fold above the detection limit within 24 hours after initiation of treatment. CRP levels were substantially elevated to a mean maximum 10-fold above the detection limit by 10 μg rhLIF/kg/d, and to approximately 20-fold above control values by 50 μg rhLIF/kg/d. CRP levels increased within 24 hours after the beginning of rhLIF administration and remained elevated throughout the 2-week rhLIF treatment period. Normal CRP levels were reestablished within 4 to 5 days after termination of rhLIF treatment. As can be seen, the three different doses of rhLIF raised CRP serum levels in a dose-dependent manner.

**Stimulation of α₁-antitrypsin levels by rhLIF.** Figure 3 depicts the effect of treatment with rhLIF on α₁-antitrypsin levels. As can be seen, α₁-antitrypsin levels began to increase within 2 to 3 days after initiation of rhLIF treatment, and reached mean maximum levels in the range of 1.5- to 2.0-fold higher than pretreatment levels by days 5 to 11. Thereafter, α₁-antitrypsin levels began to decrease reaching normal levels by days 21 to 28. It was of interest that the low dose of 2 μg rhLIF/kg/d induced a steady increase in α₁-antitrypsin levels reaching maximum levels by day 11 after initiation of treatment, whereas the higher doses of 10 and 50 μg rhLIF/kg/d stimulated a faster increase in α₁-antitrypsin levels, reaching maximum levels by day 5. As observed with CRP levels, the different doses of rhLIF elevated α₁-antitrypsin levels in a dose-dependent manner.

**Stimulation of haptoglobin levels by rhLIF.** Serum hap-
RHUF RAISES ACUTE PHASE PROTEINS AND BLOOD PLATELETS

3229

Fig 2. rhLIF treatment increases CRP levels in Rhesus monkey serum. Monkeys received rhLIF sc on the days indicated (*). The daily dose was divided into two injections. CRP levels are shown as the mean of the two animals in each group.

toglobulin levels became elevated in response to rhLIF treatment within 2 to 3 days. Mean maximum haptoglobin levels were elevated twofold at a dose level of 2 μg and 10 μg rhLIF/kg/d, compared with pretreatment values, and fourfold when a daily dose of 50 μg rhLIF/kg was administered (Fig 4). After termination of the rhLIF treatment, haptoglobin levels returned to normal within 1 week. Vehicle treatment did not change serum haptoglobin levels significantly.

Elevation of ceruloplasmin levels by rhLIF. Significant increases in ceruloplasmin levels were found at rhLIF doses of 10 and 50 μg/kg/d, but not at the lower dose of 2 μg/kg/d (Fig 5). The maximum increase in mean ceruloplasmin levels in response to the treatment with 10 or 50 μg/kg/d was in the range of 1.5- to 2-fold. Elevated ceruloplasmin levels returned to normal within 1 week after termination of treatment.

Depression of prealbumin levels by rhLIF. The levels of the negatively regulated APP prealbumin in response to rhLIF treatment are shown in Fig 6. Compared with pretreatment, prealbumin levels decreased a maximum of 1.5-fold when treated with the three doses of rhLIF during the treatment period. Serum prealbumin levels returned to normal within 3 to 4 days after termination of treatment. No major changes were seen in the prealbumin levels of monkeys receiving vehicle treatment.

Reduction of body weight by rhLIF. A significant reduction in body weight was found in monkeys treated with a daily dose of 50 μg/kg. The maximum mean body weight loss of the two animals receiving the high-dose rhLIF treatment was approximately 10%, compared with the pretreatment body weight. This occurred during the second week after termination of the rhLIF treatment. Recovery to pretreatment body weight began during the third posttreatment week. Normal body weight had not been attained by the final observation point. No behavioral changes were seen in the monkeys either during or after the rhLIF treatment period.

Effect of rhLIF on Clinical Chemistry Parameters in rhLIF-Treated Monkeys

A significant reduction in the SGPT levels of monkeys receiving a daily dose of 10 and 50 μg rhLIF/kg was observed. Within 3 days after initiation of treatment, SGPT levels began to decrease, reaching values as low as 10% of pretreatment levels at days 7 to 15 and then increased thereafter. A decline in serum cholesterol levels was only observed in the animals receiving the high-dose treatment. Cholesterol levels were reduced within 3 days of treatment by half of the pretreatment values and remained at this level throughout the observation period.

No significant changes were found throughout the rhLIF
treatment and posttreatment observation periods in the clinical chemistry parameters such as lactate dehydrogenase (LDH), glucose, urea, triglyceride, creatinine, total protein, and albumin serum levels. Also, no changes occurred in the serum calcium, magnesium, and chloride levels (results not shown).

**Serum Antibodies Against rhLIF in Monkeys**

The sera of the two monkeys receiving the high-dose rhLIF treatment of 50 µg/kg/d were tested for the development of anti-rhLIF antibodies. Both animals began to develop antibodies during the second week of rhLIF administration. In one monkey, plateau levels of approximately 10 µg IgG/mL serum were reached during the first posttreatment week, and remained at that level throughout the posttreatment observation period. In the other monkey the antibodies did not appear until the posttreatment period, where they continued to increase up until the termination of observation. The biologic test that was the induction of differentiation and the suppression of growth of the M1 mouse myeloid leukemia cell line was inhibited by 50% at dilutions ranging from 1:40 to 1:160. These data suggest that the antibodies, although binding, are for the most part nonneutralizing. From the point of view that neutralizing antibodies were found only during the posttreatment observation period, it can be assumed that the development of anti-rhLIF antibodies would not restrict the response to the rhLIF treatment. However, we cannot exclude that low levels of antibodies may have been produced while rhLIF was still being injected and these might have removed some rhLIF by complexing.

**rhLIF Treatment Does Not Elevate IL-6 Blood Levels in Monkeys**

Previously, it was found that rhIL-6 is a potent stimulator of platelet production in monkeys. Therefore, it seems logical to determine whether rhLIF treatment elevates IL-6 blood levels. As seen in Table 1, no significant increase in IL-6 blood levels could be detected either in sc-treated monkeys or in animals receiving 50 µg rhLIF/kg as an IV bolus injection. In contrast, an IV bolus injection of 100 ng endotoxin/kg raised IL-6 in blood to maximum levels several
hundred-fold above pretreatment values within 2 hours after the injection.

DISCUSSION

The results of the present study show that monkeys responded to the sc administration of various doses of rhLIF with an increase in several APPs in a dose-dependent manner. In vitro, the principal hepatocyte-regulating activities have been identified as IL-6, IL-1, tumor necrosis factor α (TNFα), and LIF. Monkeys responded to the high-dose rhLIF treatment with a more than 20-fold increase in CRP levels. The lower doses of rhLIF induced a minor elevation of serum CRP levels. In contrast to this finding, α1-antitrypsin, haptoglobin, and ceruloplasmin levels were increased to maximum levels twofold to threefold above pretreatment values. A substantial decrease in the negatively regulated APP prealbumin was found only in animals receiving the mid- and high-dose rhLIF treatment. Similar changes in the APP were found to be induced in monkeys by rhIL-6 administration. However, further experiments must be performed to determine whether both cytokines are able to induce the whole set of APP or whether differences exist.

rhLIF treatment was found to induce a 1.5- to 2-fold increase in blood platelet counts. Although the magnitude of the platelet elevation was similar to that observed with rhIL-6, the onset of the increase occurred later. The platelet counts in monkeys receiving rhIL-6 began to increase by days 4 to 5 after initiation of treatment, whereas the rhLIF-treated monkeys only showed an elevation in platelet counts at the end of the 14-day treatment. The observation was also made that in rhIL-6-treated monkeys, most of the APP platelet levels coincided with the maximum platelet counts. However, in the monkeys receiving rhLIF treatment, the APP platelet levels were reached on day 4 and lasted to day 14 of the treatment, while maximum platelet counts were reached on day 11 and lasted to day 20 of rhLIF treatment. It is unclear what mechanism is responsible for the induction of the increase in platelet counts. In this regard recent work is of interest, showing secretion of IL-6 by LIF-stimulated murine myeloid M1 leukemia cells. However, the possibility that the rhLIF-induced elevation of platelet counts is modulated via stimulation of IL-6 production can be excluded in vivo because we were not able to detect a significant increase in blood IL-6 levels in monkeys receiving rhLIF either sc or as an IV bolus injection. In the mouse, the administration of rmLIF resulted in a twofold to fivefold increase of megakaryocytes in the spleen and their

Table 1. Effect of rhLIF Administration on IL-6 Blood Levels in Monkeys

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Monkey No.</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhLIF sc*</td>
<td>1</td>
<td>3.1</td>
<td>4.5</td>
<td>3.7</td>
<td>2.9</td>
<td>2.1</td>
<td>2.8</td>
<td>3.6</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.5</td>
<td>3.2</td>
<td>3.9</td>
<td>2.1</td>
<td>3.6</td>
<td>4.7</td>
<td>5.3</td>
<td>3.7</td>
</tr>
<tr>
<td>rhLIF IV†</td>
<td>3</td>
<td>1.9</td>
<td>1.3</td>
<td>2.6</td>
<td>3.7</td>
<td>2.9</td>
<td>1.3</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.2</td>
<td>5.7</td>
<td>4.7</td>
<td>2.0</td>
<td>4.0</td>
<td>3.7</td>
<td>5.1</td>
<td>2.9</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>5</td>
<td>1.6</td>
<td>172.3</td>
<td>520.3</td>
<td>460.0</td>
<td>372.2</td>
<td>273.3</td>
<td>140.2</td>
<td>76.3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.2</td>
<td>276.2</td>
<td>730.1</td>
<td>698.1</td>
<td>572.3</td>
<td>178.2</td>
<td>97.2</td>
<td>20.3</td>
</tr>
</tbody>
</table>

* rhLIF was administered at a daily dose of 50 µg/kg. The daily dose was divided into two sc injections. Monkeys were pretreated with the high dose of rhLIF for 6 days.
† A single IV bolus injection of 50 µg rhLIF was administered.
‡ Endotoxin (Salmon abortus equi) was administered at 100 ng/kg as a single IV bolus injection.
significant elevation (of a lesser magnitude) in the BM. 3 In the monkey, rhLIF treatment induced no changes in the number and size of megakaryocytes in the BM. Because of the slow response of rhLIF administrations on the increase in blood platelet counts in monkeys, it is unlikely that this effect is caused by increased thrombocyte DNA polyploidization, reduced thrombocyte destruction, or an accelerated release of platelets into the circulation.

The daily injection of rmLIF into mice failed to produce the characteristic neutrophil leukocytosis seen in the murine model, where the animals were engrafted with leukemic cells carrying the gene for LIF, thus producing excessive amounts of murine LIF. 6,25 In the monkey study, rhLIF treatment had no influence on the total and differential blood leukocyte counts.

No changes were observed in the number of progenitor cells of the different lineages in the blood of LIF-treated monkeys. Elevated circulating progenitor cell levels were seen in monkeys treated with rhIL-3, rhGM-CSF, 26 and, to a minor degree, rhIL-6. 19 Therefore, it can be suggested that in vivo rhLIF has no major influence on the progenitor cell pool. However, additional studies that include cultures of hematopoietic cells from the BM of rhLIF-treated animals are necessary to clarify the question. In vitro, LIF was found to inhibit the basal bone resorption, probably by acting at the level of osteoblasts. 27 Mice engrafted with cells producing high levels of LIF developed calcification in heart and skeletal muscle, and showed excess formation of new bone. 24 Also, the administration of rmLIF to normal mice, even at low doses, elevated serum calcium levels. 6 However, in the present study, we were not able to show changes in serum calcium levels throughout the treatment or the post-treatment observation periods. As the capacity of rhLIF and other cytokines to raise serum calcium levels has been considered critical for promoting new bone formation, 24 the possibility exists that LIF does not have this biologic activity in nonhuman primates. Another possibility is that the bone-modulating activity of rhLIF can be found only at higher doses than those used in the present study.

Body weight loss, caused mainly by a reduction in subcutaneous and abdominal fatty tissue, and the development of a hyperactive state were observed both in mice engrafted with the LIF-producing hematopoietic cell line (FDC-P1) and in mice injected with rmLIF on a daily basis. 6,24 In our monkey study, a substantial body weight loss was observed only in animals receiving the high-dose rhLIF treatment. In contrast to the rmLIF-treated mice, monkeys receiving rhLIF did not show changes in their activity and behavior.

In conclusion, we have shown that rhLIF is able to increase platelet counts and several APP levels in nonhuman primates. Further studies will show whether LIF either alone or in combination with other cytokines such as IL-3 and/or IL-6, can be used in the treatment of thrombocytopenia in humans.

REFERENCES


22. Perlmutter DH, Dinarello CA, Funsal PJ, Cohen HR: Ca-


Recombinant human leukemia inhibitory factor induces acute phase proteins and raises the blood platelet counts in nonhuman primates

P Mayer, K Geissler, M Ward and D Metcalf