Recombinant Human Interleukin-6 Induces a Rapid and Reversible Anemia in Cancer Patients

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Initial studies have shown that recombinant human interleukin-6 (rhIL-6) induces anemia. Until now, the pathophysiological mechanism of this induced anemia has been unknown. To unravel the underlying mechanism, we examined 15 cancer patients receiving rhIL-6 as an antitumor immunotherapy in a phase II study. rhIL-6 was administered subcutaneously at 150 μg once daily for 6 consecutive weeks. Various hematologic and biochemical parameters were measured weekly during rhIL-6 treatment and 4 weeks after rhIL-6 discontinuation. To determine plasma volume and red blood cell (RBC) volume, radioisotope dilution assays with labeled autologous RBCs and with human serum albumin were performed during rhIL-6 treatment and before rhIL-6 administration. Hemoglobin levels decreased (mean change ± SE) 7% ± 1.5% within 3 days after the start of rhIL-6 therapy (P < .0001) and 19% ± 2% at week 4. Levels had normalized after follow-up. The plasma volume increased 78% ± 5% during the first week of rhIL-6 administration (P < .0003), whereas RBC volume remained unaffected. The mean RBC corpuscular volume remained unchanged for 2 weeks and then began to decrease slowly, reaching its nadir at week 6 (5% ± 1%; P < .01). Serum iron levels decreased 65% ± 12% at week 4 (P < .002) and then returned to initial baseline values. Erythropoietin levels increased rapidly up to 68% at week 3 (P < .0001) and had normalized 4 weeks after rhIL-6 therapy. Levels of serum albumin, prealbumin, and transferrin decreased (P < .0001, P < .003, and P < .0001, respectively), whereas levels of serum amyloid A (P < .0003), C-reactive protein, haptoglobin, and α1-antitrypsin (P < .0001) increased during rhIL-6 treatment. All levels returned to pretreatment values after discontinuation of rhIL-6. No alterations in reticulocyte counts, serum lactate dehydrogenase levels, and bilirubin levels were observed. A 6-week regimen of subcutaneous rhIL-6 results in a rapid dilution anemia, caused by an acute and significant increase in plasma volume and followed by hypoferremia. This anemia is reversible after the cessation of rhIL-6 treatment.

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INTERLEUKIN-6 (IL-6) is a 26-kD protein released by fibroblasts, T lymphocytes, endothelial cells, monocytes, and keratinocytes during inflammatory responses. Previous studies have shown that IL-6 has pluripotent activities, including the stimulation of the monocytic lineage, the induction of megakaryopoiesis and the hepatic acute phase response, as well as the modulation of T- and B-cell responses. Based on experimental and preclinical data, it appears that IL-6 is also capable of mediating direct and indirect tumor cell destruction and that it is potentially important for the immunotherapy of cancer. However, for certain tumor types IL-6 may also act as a stimulatory factor.

Using DNA cloning technology, large quantities of recombinant human IL-6 (rhIL-6) can be produced for investigational purposes. Recently, rhIL-6 became available for clinical use. Its pleiotropic properties have initiated phase I-II clinical trials with rhIL-6 alone as an antitumor agent or as a stimulator of thrombopoiesis after chemotherapy.

Side effects of rhIL-6 included the appearance of a normochromic normocytic anemia, as shown by recent reports on rhIL-6 treatment in cancer patients. Van Gameren et al found a dose-dependent decrease in hemoglobin (Hb) levels in all patients within 3 days since the initiation of rhIL-6 administration.

To determine the pathophysiological mechanism responsible for the rhIL-6–associated anemia, a series of hematologic and serologic investigations was performed in cancer patients receiving rhIL-6 as part of a phase II study of antitumor immunotherapy for 6 consecutive weeks. In addition, plasma volume and red blood cell (RBC) volume were measured serially, ie, before rhIL-6 administration, and on day 8 of rhIL-6 treatment.

MATERIALS AND METHODS

Patients. All patients had a histologically confirmed diagnosis of progressive metastatic renal cell cancer or malignant melanoma and participated in two separate multicenter phase II studies of treatment with subcutaneous (SC) rhIL-6. Inclusion criteria were age between 18 and 75 years, a performance status ≤1 (World Health Organization [WHO] scale), and adequate functions of liver (serum transaminase levels, <1.5 × 10⁶ U/L; γ-glutamyl transpeptidase, <115 U/L), kidneys (serum creatinine level, <180 μmol/L), and bone marrow (Hb, ≥120 g/L; leukocytes, ≥3 × 10⁹/L; platelet count, ≥100 × 10⁹/L). Patients with nonmeasurable lesions only as well as patients with brain metastases were excluded from the study. No other concomitant malignancies apart from the tumor were apparent, and no patient received any immunosuppressive therapy. None of the patients had an active viral infection or an autoimmune disease. Chemotherapy or any investigational compound was discontinued 4 weeks before entry into the study. The studies were performed in two strata, ie, with or without previous immunotherapy. The study protocols were approved by the Medical Ethical Committee of the University Hospital Groningen, Groningen, The Netherlands. All patients gave informed consent before treatment.

Study design. To assess initial tolerability and safety, patients were hospitalized during the first 7 days of rhIL-6 administration. If no major adverse events occurred, patients were treated on an outpatient basis thereafter. The treatment consisted of an established dose of 150 μg rhIL-6 once daily SC for 6 consecutive weeks. Escherichia coli–derived rhIL-6 (10⁴ U/mg protein) was provided...
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by Sandoz Pharmaceutical Ltd (Basel, Switzerland). For SC injection, 2-mL vials containing 150 μg lyophilized rhIL-6 were reconstituted with 1 mL of sterile water.

**Toxicity assessment.** Toxicity was scored according to the WHO criteria. A careful history with emphasis on bleedings and menorrhagias was recorded once weekly. Blood pressure, pulse rate, temperature, and body weight were measured every week. To prevent fever or influenza-like symptoms, each patient received acetaminophen with a maximum of 3 g per day. No nonsteroidal, anti-inflammatory drugs were prescribed.

**Hematologic and biochemical examinations.** For hematologic and biochemical examinations, blood samples were obtained before the initiation of rhIL-6 therapy (zero time), during rhIL-6 administration (days 3, 8, 15, 22, 29, 36, and 43), and 4 weeks after the cessation of rhIL-6 treatment. Parameters measured included full blood cell counts, Hb, mean RBC corpuscular volume (MCV), differential leukocyte counts, and serum levels of creatinine, urea, lactate dehydrogenase (LDH), alkaline phosphatase, gamma glutamyl transpeptidase (GGT), sodium, potassium, calcium, total protein, albumin, glucose, total cholesterol, and iron. On days 3, 22, and 43 and 4 weeks after discontinuation of rhIL-6 treatment additional blood samples were drawn for measuring serum levels of erythropoietin (EPO), IgG, IgA, IgM, vasopressin (AVP), total renin, active renin, and acute-phase proteins, ie, C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin, transferrin, prealbumin, and α-1-antitrypsin. EPO levels were measured by a radioimmunoassay (RIA; Sorin Biomedica, Stillwater, MN). Levels of IgG, haptoglobin, transferrin, prealbumin, and α-1-antitrypsin were measured by a Behring nephelometer (Behringwerke AG Diagnostica, Marburg, Germany). CRP (normal value, <2 mg/L) and SAA (normal value, <3 mg/L) were determined using enzyme-linked immunosorbent assays (ELISA), whereas AVP as well as total and active renin were measured using radioimmunoassay kits (Nichols Institute Diagnostics SA, Geneva, Switzerland).

**Radioisotope dilution assay.** Before rhIL-6 administration (zero time) and on day 8 of rhIL-6 treatment, RBC volume and plasma volume were measured according to the recommended methods, with some minor modifications, to increase the accuracy of the method. The technique is based on a simultaneous dilution analysis of radioactive 51Cr-labeled autologous RBCs and human serum albumin. For the RBC labeling, 51Cr (1.0 MBq) was used; thereafter, 125I human serum albumin (0.25 MBq, containing <1% of free iodine) was mixed with the labeled RBC suspension. When the patient had rested for 15 minutes in a recumbent position, 20 mL of this mixture was injected intravenously. The patient remained in the same position for 60 minutes while 10 mL blood samples were drawn from a contralateral arm vein and collected in heparinized tubes at t = 0, 10, 20, 30, 40, 50, and 60 minutes. Radioactivity in standards and blood specimens were measured using the 270 to 370 keV range for 51Cr energy windows of 251Cr and the 20 to 70 keV window for 125I radioactivity with correction for low energy 51Cr radioactivity measured in this energy window. The time zero sample was used for subtraction of eventual radioactivity from previous studies. The radioactivity of 51Cr and 125I measured in the samples t = 10, 20, 30, 40, 50, and 60 minutes was used to extrapolate to zero time to calculate RBC volume and plasma volume. Extrapolation was necessary to increase the accuracy of the plasma volume measurement, because in normal subjects [125I]-albumin leaks from the circulation at a rate of about 4%/h. Following this procedure, the coefficient of variance was 3%. In the same manner, spleenic uptake of 51Cr-labeled RBCs was measured externally to evaluate the possibility of increased RBC pooling in the spleen. At zero time (90 minutes after administration of the tracer) and on days 3, 5, and 8 during rhIL-6 therapy, a probe was positioned over the splenic area. Careful reposition of the probe was performed with the help of skin markers. Counts in the 270 to 370 keV range were collected for 10 minutes. After background correction and correction for decay, data were expressed as the percentage of the radioactivity measured over the splenic area at zero time. An upsloping curve was considered as evidence for increased RBC pooling in the spleen.

**RESULTS**

**Patient characteristics.** Fifteen patients (7 with renal cell cancer and 8 with malignant melanoma) were entered in this study and were evaluated for clinical, hematologic, and biochemical parameters. Patient characteristics are summarized in Table 1. The last 11 patients underwent additional measurements of RBC volume and plasma volume. Premature interruption of rhIL-6 administration occurred in 2 patients because of fulminant tumor progression at weeks 4 and 6, respectively. Before the initiation of rhIL-6 therapy all patients had normal levels of Hb (normal value for men, 135 to 180 g/L; normal value for women, 120 to 160 g/L) and serum iron (normal value for men, 14 to 32 μmol/L; normal value for women, 10 to 28 μmol/L).

**Toxicity.** The clinical toxicity of SC rhIL-6 administration mainly consisted of fever and chills associated with nausea and vomiting. All 15 patients developed fever up to WHO grade I-II, which occurred within a few hours after the rhIL-6 injection and generally resolved before the following injection. All patients had chills starting 30 minutes after the injection and disappearing within approximately 15 to 30 minutes. A daily oral dose of 3 g of acetaminophen partially alleviated these flu-like symptoms. All patients experienced loss of appetite during the rhIL-6 course, although no changes in body weight were observed. Three patients complained of transient nausea and vomiting, which did not require antiemetic therapy. All patients had local erythema at the injection sites, which subsided after rhIL-6 treatment was discontinued. Progressive fatigue was reported by all patients and often required 2 to 4 weeks for resolution. No history of blood loss and/or menorrhagias was reported. One patient required 3 U of RBCs at week 3, whereas another patient required 4 U of RBCs at week 5 of rhIL-6 treatment. Both patients were excluded from further evaluation for anemia from the moment of RBC transfusion.

**Hematologic effects.** All 15 patients developed anemia, which could not be explained by the number of blood samples drawn from each patient before and during rhIL-6 treatment. The anemia was illustrated by a rapid and significant increase in plasma volume and RBC volume. Extrapolation was necessary to increase the accuracy of the plasma volume measurement, because in normal subjects [125I]-albumin leaks from the circulation at a rate of about 4%/h. Following this procedure, the coefficient of variance was 3%. In the same manner, spleenic uptake of 51Cr-labeled RBCs was measured externally to evaluate the possibility of increased RBC pooling in the spleen. At zero time (90 minutes after administration of the tracer) and on days 3, 5, and 8 during rhIL-6 therapy, a probe was positioned over the splenic area. Careful reposition of the probe was performed with the help of skin markers. Counts in the 270 to 370 keV range were collected for 10 minutes. After background correction and correction for decay, data were expressed as the percentage of the radioactivity measured over the splenic area at zero time. An upsloping curve was considered as evidence for increased RBC pooling in the spleen.

**Statistics.** The two-tailed, paired Student's t-test was used for statistical analysis. P values < .05 were considered significant.
The MCV remained unchanged for 2 weeks and then decreased very slowly, with minimum values at week 6. After rhIL-6 treatment, the MCV remained significantly lower than initial baseline values for at least 4 weeks (mean ± SE; day 0, 85.5 ± 2.8 fL; week 4, 81.7 ± 2.5; P < .01; week 10, 82.9 ± 2.5; P < .006). As shown in Fig 2, the anemia was accompanied by a significant decrease in serum iron levels, with the lowest values occurring at week 4 (mean ± SE; day 0, 11.1 ± 1.9 μmol/L; week 4, 2.9 ± 0.4; P < .002). All levels appeared to return to initial baseline values before the end of rhIL-6 treatment. Serum EPO levels increased, with peak levels at week 3 (mean ± SE; day 0, 17.83 ± 1.80 IU/L; week 3, 30.00 ± 2.40; P < .001), and returned to pretreatment values after rhIL-6 treatment discontinuation. No increase in (for anemia corrected) reticulocyte counts was observed during or after rhIL-6 administration. Serum LDH and bilirubin levels both remained unchanged.

The platelet count increased after an initial decrease at day 3, with maximum values at week 3 (mean ± SE; day 0, 327 × 10^9/L; day 3, 306 ± 18; P < .03; week 3, 319 ± 28; P < .001). Levels returned to pretreatment values after cessation of rhIL-6 (Fig 3).

No consistent changes in total leukocyte numbers were seen. After an initial decrease in IgG levels during the first week of rhIL-6 administration (mean ± SE; day 0, 16.70 ± 1.37 g/L; week 1, 15.42 ± 1.30; P < .006), a slight increase occurred (not significant [NS]). No significant alterations in IgA and IgM levels were observed.

Biochemical effects. Serum creatinine levels remained stable for 1 week, but decreased significantly afterwards, with the nadir occurring at week 2 (mean ± SE; day 0, 92 ± 5 μmol/L; week 2, 84 ± 5; P < .02). Serum levels of urea showed a slight decrease during the first 2 weeks (mean ± SE; day 0, 5.9 ± 0.5 μmol/L; day 3, 5.1 ± 0.4; P < .008; week 1, 5.3 ± 0.4; P < .02; week 2, 5.2 ± 0.4; P < .04), then all returned to initial baseline values before discontinuation of rhIL-6 treatment. After an early decrease on day 3 (mean ± SE; day 0, 141 ± 0.5 mmol/L; day 3, 140 ± 0.5; P < .05), the sodium level returned to pretreatment values and remained stable during the following weeks of rhIL-6 treatment. No alterations in serum levels of AVP, total renin, and active renin were noted during or after rhIL-6 administration.
Table 2. Effect of rhIL-6 on Acute-Phase Proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Baseline</th>
<th>Maximum/Minimum</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/L)</td>
<td>43 ± 1</td>
<td>37 ± 1</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Prealbumin (g/L)</td>
<td>0.23 ± 0.03</td>
<td>0.14 ± 0.01</td>
<td>&lt;.003</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>2.71 ± 0.15</td>
<td>2.11 ± 0.11</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Haptoglobin (g/L)</td>
<td>4.49 ± 0.46</td>
<td>7.48 ± 0.50</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>α-1-Antitrypsin (g/L)</td>
<td>4.00 ± 0.27</td>
<td>6.13 ± 0.36</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>51 ± 18</td>
<td>263 ± 38</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>SAA (mg/L)</td>
<td>54 ± 27</td>
<td>387 ± 69</td>
<td>&lt;.003</td>
</tr>
</tbody>
</table>

GGT increased with peak levels occurring at week 4 (mean ± SE: day 0, 64 ± 18 U/L; week 4, 141 ± 42; P < .02) and normalization after rhIL-6 therapy. No significant changes in alkaline phosphatase were observed, although mean values tended to increase during rhIL-6 administration, with peak levels occurring at week 4. The total serum protein levels remained unaffected.

Effects on acute-phase proteins. The effects of rhIL-6 on acute-phase proteins are summarized in Table 2. A significant decrease in serum albumin levels was found on day 3 of rhIL-6 therapy, with further reduction until week 2. After discontinuation of rhIL-6 treatment, levels returned to pretreatment values. In addition, a significant decrease in levels of prealbumin and transferrin developed, with minimum values occurring at week 1 followed by a slow recovery. At follow-up, all levels had normalized (Fig 4). Haptoglobin and α-1-antitrypsin levels increased significantly during the first week and reached maximum values at week 3. These levels remained stable during the following weeks of rhIL-6 therapy and had returned to initial baseline values at follow-up (Fig 5).

Radioisotope findings. Radioisotope dilution studies were performed in 11 patients at study entry and on day 8 of rhIL-6 therapy. An 18% ± 5% increase in plasma volume was observed 1 week after rhIL-6 initiation (mean ± SE; day 0, 47 ± 4 ml/kg; day 8, 55 ± 4; P < .003), whereas RBC volume did not change (mean ± SE; day 0, 25 ± 2 ml/kg; day 8, 24 ± 2; NS). Between days 0 and 8, no significant increase in splenic uptake of 51Cr-labeled autologous RBCs was observed (data not shown).

DISCUSSION

An earlier report on SC rhIL-6 treatment in nonhuman primates showed a slight normochromic normocytic anemia
that reversed after cessation of rhIL-6 treatment.\textsuperscript{36} In addition, recent reports on SC rhIL-6 administration in cancer patients also showed the development of a normochromic normocytic anemia.\textsuperscript{30,31} Potential reasons for the observed anemia were considered to be the induction of the hepatic acute-phase response, alterations in RBC production, sequestration, suppression of erythroid burst-forming units (BFU-E),\textsuperscript{30} and/or an increase in plasma volume.\textsuperscript{31}

In the present study, we established the underlying mechanism of the rhIL-6-associated anemia in cancer patients receiving rhIL-6 as an antitumor immunotherapy for 6 consecutive weeks. Within 3 days since the start of rhIL-6 therapy, a rapid and significant decrease in the Hb level was observed in all patients, reaching its nadir at week 4 and returning to pretreatment values within 4 weeks after rhIL-6 treatment discontinuation. Reduction of Hb could be ascribed neither to the volume of blood collected during rhIL-6 administration nor to hemolysis, because no alterations in reticulocyte counts, serum LDH, and bilirubin levels were seen.

Because the anemia occurred so rapidly, it was considered that it might reflect a shift in RBC volume or plasma volume or an increase in sequestration of RBCs in the spleen. Serial isotope dilution assays in our patients showed a significant increase in plasma volume without changes in RBC volume and splenic uptake, indicating that the rapid increase in the Hb level during the initial phase could be ascribed to hemodilution. These findings are in agreement with clinical data reported by Atkins et al.\textsuperscript{31} Hemodilution might also explain the initial decrease in platelet counts and serum albumin levels.

At least three hypothetical explanations for hemodilution could be postulated, ie, an increase in plasma colloid osmotic pressure, renal impairment, and/or the release of AVP by the hypothalamus.

Because serum albumin levels decreased 14\%, one may expect a concurrent decrease in plasma colloid osmotic pressure, leading to extravasation of fluid, with signs of edema and weight gain. In our patients, edema and weight gain were absent. On the contrary, a remarkable increase in the Hb level during the initial phase could be ascribed to hemodilution. This anemia is reversible after cessation of rhIL-6 treatment.

Besides hemodilution, additional mechanisms might also operate, because the anemia progressed further during the following weeks of rhIL-6 administration. One mechanism might be a change in the ferrokinetics. It has recently been shown in rats that rhIL-6 induces a rapid hypoferremia by mobilizing the transferrin-bound serum iron to hepatocytes by means of transferrin receptors. Furthermore, rhIL-6 has been shown to augment ferritin synthesis as well as to decrease transferrin synthesis.\textsuperscript{34} Our finding that both serum iron and transferrin levels decreased significantly during rhIL-6 therapy is in agreement with these animal data. However, because the turnover of RBCs is approximately 4 months, hypoferremia may be needed for an adequate period of time to induce anemia. In our study this is probably reflected by a slow decrease in the MCV, which is not apparent before week 3 and finally reaches its nadir at week 6.

Additionally, increased \textalpha{}-1-antitrypsin levels might compete with the binding of transferrin to its ligand,\textsuperscript{38} as shown for human erythroleukemic cells (K562). Moreover, in vitro evidence was obtained that \textalpha{}-1-antitrypsin might inhibit growth and proliferation of the BFU-E. In our study, no iron staining of the bone marrow was performed. However, a previous trial of rhIL-6 treatment by our group did not show any changes in BFU-E numbers after 7 days of rhIL-6 administration.\textsuperscript{30} The significant increase in serum EPO levels despite no significant change in RBC volume seems to confirm an in vitro finding of Faquin et al\textsuperscript{40} that rhIL-6 may stimulate EPO production.

In conclusion, we have shown that a 6-week regimen of SC rhIL-6 results in a rapid dilution anemia caused by an acute and significant increase in plasma volume and followed by hypoferremia. This anemia is reversible after cessation of rhIL-6 and seems to be of potential clinical importance. Because no significant change in RBC volume has been shown in this study, in the future physicians should perhaps not be as concerned with the rapid decrease in Hb levels, and they may need to rethink their criteria for administering RBC transfusions to patients receiving rhIL-6.

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