Recombinant human interleukin-6 (rhIL-6) is a pluripotent cytokine with proinflammatory, antitumor, and growth factor effects. Clinical investigations of rhIL-6 either alone as immunotherapy or as a colony-stimulating factor in conjunction with chemotherapy have shown a dose-dependent, rapid onset, and largely reversible decrease in venous hematocrit levels. In an effort to determine the mechanism for the rhIL-6-associated anemia, we measured red blood cell volume serially in patients receiving rhIL-6 at either 30 μg/kg/day as a 120-hour continuous intravenous infusion (renal cell carcinoma) or 100 μg/kg/d intravenously over 1 hour for 5 days (melanoma) as part of two separate phase II trials. Radioisotope dilution assays with 51Cr-labeled autologous red blood cells and hemolysis screens were performed on day 1 before the initiation of therapy and on day 5 shortly before the end of therapy. In the 6 patients studied, the mean decrease in hemoglobin concentration was 1.9 ± 0.94 g/dL.

The mean decrease in the hematocrit level was 6% ± 2% and the mean increase in total blood volume was 731 ± 337 mL. These changes were explained by a mean decrease in red blood mass of 106 ± 109 mL and a mean increase in plasma volume of 743 ± 289 mL. The decrease in red blood cell mass was largely explained by phlebotomy during the hospitalization, but was not statistically significant (paired t-test, \( P = .06 \)). All other changes were statistically significant (\( P < .05 \)). Simple regression analysis indicated that the decrease in hematocrit level and increase in plasma volume were related (\( y = -1.78 - .0066x; R = - .74 \)). Measurements of lactate dehydrogenase, bilirubin, haptoglobin, and reticulocyte counts and serial stool hemoccults did not indicate hemolysis or blood loss. We conclude that the anemia caused by IL-6 is caused by an increase in plasma volume.

INTERLEUKIN-6 (IL-6) IS A PLURIPOTENT CYTOKINE WITH PROINFLAMMATORY, HEMATOPOIETIC, AND IMMUNOMODULATORY EFFECTS. IN PRECLINICAL TRIALS, IL-6 HAS SHOWN BOTH DIRECT AND INDIRECT ANTITUMOR EFFECTS AND HAS BEEN ABLE TO INDUCE HYPERBILIRUBINEMIA, CONFOCAL MUSCLE WEAKNESS, AND ARRHYTHMIAS. LABORATORY EFFECTS HAVE INCLUDED HYPERBILIRUBINEMIA, CONFUSION, PROXIMAL MUSCLE WEAKNESS, AND ATRIAL ARRHYTHMIAS. LABORATORY EFFECTS HAVE INCLUDED SERUM C-REACTIVE PROTEIN AND CORTISOL ELEVATIONS, OCCASIONAL HEPATIC TRANSAMINITIS, A DOSE-DEPENDENT THROMBOCYTOSIS, AND ANEMIA. MODEST ANTIMUTU COR EFFECTS HAVE BEEN OBSERVED IN THE FEW PHASE II TRIALS REPORTED TO DATE.

The anemia associated with rhIL-6 administration is rapid in onset, dose-dependent, and progressive, but is quickly reversible after the cessation of rhIL-6 therapy. Decreases in hemoglobin level, hematocrit level, and red blood cell (RBC) number of 10% to 20% of baseline have been routinely observed. This anemia was particularly problematic when rhIL-6 was used as a hematopoietic growth factor, with rhIL-6 administration actually increasing transfusion requirements for patients with aplastic anemia or receiving concomitant cytotoxic chemotherapy. Weber et al reported the anemia to be normocytic and unaccompanied by signs of hemolysis or alterations in the number or appearance of bone marrow erythroid precursors. Vamguren et al further reported that the anemia was characterized by the decrease in serum iron, an increase in ferritin, and an increase in erythropoietin without reticulocytosis or change in burst-forming unit-erythroid (BFU-E) numbers. Both investigators speculated that the anemia was due to sequestration.

Srinivasan et al observed a similar reversible anemia in monkeys receiving 5 to 30 μg/kg/day of rhIL-6 subcutaneously. Additional investigations in these animals showed a decrease in the proportion of nucleated RBCs in the bone marrow as the total cellularity increased and a decrease in serum iron levels and iron-binding saturation. Circulating reticulocyte counts, serum bilirubin, and haptoglobin levels remained unchanged. These investigators also postulated that the anemia was secondary to RBC sequestration.

In an effort to determine the etiology of the rhIL-6-associated anemia, we investigated the effects of rhIL-6 on plasma and total blood volume and RBC mass in patients receiving rhIL-6 immunotherapy as part of two phase II trials.

MATERIALS AND METHODS

Treatment. Patients received Escherichia coli-derived, nonglycosylated rhIL-6 (Sandoz Pharmaceutical Corp, East Hanover, NJ) at either 30 μg/kg/d as a 120-hour continuous intravenous (IV) infusion (renal cell carcinoma) or 100 μg/kg/d IV over 1 hour for 5 days (melanoma) as part of two separate phase II trials performed by the Cytokine Working Group. The methods and results of these trials have been previously described. The rhIL-6 protocols and blood volume studies were approved by the Institutional Review Board at the New England Medical Center and all patients gave written informed consent for both studies.
Blood volume studies. Radioisotope dilution using \(^{51}\)Cr-labeled autologous RBCs was used to measure total blood volume, plasma volume, and RBC mass. These studies were performed on day 1 immediately before the initiation of rhlL-6 therapy and on day 5 shortly before the completion of rhlL-6 therapy. The blood volume studies were performed according to published techniques.\(^{21}\) In brief, \(^{51}\)Cr (New England Nuclear, Boston, MA) was used to label autologous RBCs. Ascorbic acid was used to stop the reaction.\(^{25}\) Nine milliliters of RBCs was injected IV and blood samples were drawn after 20 minutes from a different site. On day 5, residual background counts were subtracted from those obtained after injection and equilibration. According to convention, a correction factor of 0.91 was used in the calculation of total blood volume and a factor of 0.96 was used to correct for trapped plasma in calculating RBC mass. Plasma volume was calculated by subtracting RBC mass from total blood volume.

Additional evaluations. Serial serum lactate dehydrogenase (LDH) and bilirubin levels and day-5 haptoglobin levels were obtained. Reticulocyte counts on day 0 and day 5 were obtained in 2 of 6 patients. Stool specimens were routinely tested for occult blood.

Statistics. Means, standard deviations, paired t-tests, and linear regression analysis was performed using Statgraphics, version 4.0 (Statistical Graphics Corp, Rockville, MD).

RESULTS

Patient and protocol characteristics. Six patients, 2 with melanoma and 4 with renal cell cancer, were studied. Patient characteristics are displayed in Table 1. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0-1 and no patient had received prior systemic therapy. Standard phlebotomy volumes accompanying these protocols are shown in Table 2. On average, patients were phlebotomized 150 mL of whole blood (approximately, 50 to 60 mL of RBCs). No patient received RBC transfusions during the course of therapy.

RBC volume determinations. Serial changes in mean hemoglobin concentrations are displayed in Fig 1. As has been previously reported, mean hemoglobin concentrations decreased steadily during therapy to about 80% of baseline on day 6 and largely recovered by day 8, 48 to 72 hours after the completion of therapy. Mean changes in hemoglobin, hematocrit, RBC mass, total blood volume, and plasma volume determinations on day 5 relative to day 1 are displayed in Table 3. Four of six patients began the study with a decreased RBC mass and an increased plasma volume compared with gender-specific normals. There was a mean decrease in hemoglobin and hematocrit levels of approximately 16% and a mean decrease in RBC mass of 106 ± 109 mL (6% ±6%). In contrast, there was a mean increase in total blood volume and plasma volume of 14% ± 6% and 22% ± 8%, respectively. These data are shown graphically in Fig 2. Stool hemoccults were repeatedly negative, serum LDH levels decreased an average of 44% ± 22%, changes in serum bilirubin were small (0.1 ± 0.37 mg/dL), and haptoglobins measured on day 5 were within the normal range. Reticulocyte counts were performed in 2 patients and were 1.1% for both patients on day 1 and 0.9% and 1.8% on day 5. Statistical analysis (paired t-test) showed significant differences in hemoglobin level, hematocrit level, total blood volume, and plasma volume (\(P < .05\)). The mean decline in RBC mass was largely explained by phlebotomy and approached, but did not reach, statistical significance (\(P = .06\)). Simple regression analysis using hematocrit level and plasma volume as variables gave the following equation: y = -1.78 - .0066 X; \(R = -.74\) (Fig 3).

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>PS</th>
<th>Disease</th>
<th>Prior Tx</th>
<th>Baseline Hgb/Hct</th>
</tr>
</thead>
<tbody>
<tr>
<td>65/M</td>
<td>0</td>
<td>RCCA</td>
<td>Surgery</td>
<td>12.2/37.1</td>
</tr>
<tr>
<td>60/M</td>
<td>0</td>
<td>RCCA</td>
<td>Surgery/RT</td>
<td>13.9/42.4</td>
</tr>
<tr>
<td>49/F</td>
<td>0</td>
<td>RCCA</td>
<td>Surgery</td>
<td>13.6/42.6</td>
</tr>
<tr>
<td>48/M</td>
<td>1</td>
<td>RCCA</td>
<td>Surgery</td>
<td>13.0/39.6</td>
</tr>
<tr>
<td>55/F</td>
<td>0</td>
<td>Mel</td>
<td>Surgery</td>
<td>14.0/44.3</td>
</tr>
<tr>
<td>39/F</td>
<td>1</td>
<td>Mel</td>
<td>Surgery</td>
<td>12.2/37.3</td>
</tr>
</tbody>
</table>

Abbreviations: RCCA, renal cell carcinoma; Mel, melanoma; PS, performance status; Tx, therapy; Hgb, hemoglobin level; Hct, hematocrit level; RT, radiation therapy.

Table 2. Phlebotomy Volumes

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Volume (mL)*</th>
</tr>
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<tbody>
<tr>
<td>Routine</td>
<td>50</td>
</tr>
<tr>
<td>IL-6 protocol</td>
<td>30</td>
</tr>
<tr>
<td>Anemia evaluation</td>
<td>70</td>
</tr>
<tr>
<td>Total blood volume</td>
<td>150</td>
</tr>
<tr>
<td>~RBC volume</td>
<td>50-60</td>
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</tbody>
</table>

* Days 1 through 5.

Table 3. Results of Blood Volume Determinations

<table>
<thead>
<tr>
<th>Test</th>
<th>Absolute</th>
<th>Mean Change</th>
<th>(P Value^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb</td>
<td>-1.9 ± .94 g/dL</td>
<td>-16 ± 8</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Hct</td>
<td>-6 ± 2%</td>
<td>-15 ± 5</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>RBC mass</td>
<td>-106 ± 109 mL</td>
<td>-6 ± 6</td>
<td>.06</td>
</tr>
<tr>
<td>Blood volume</td>
<td>731 ± 337 mL</td>
<td>+14 ± 6</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Plasma volume</td>
<td>743 ± 289 mL</td>
<td>+22 ± 8</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

Simple regression analysis: Hct and plasma volume correlation coefficient, \(R = -.74\).

* Paired t-test.
DISCUSSION

A transient but significant anemia has been routinely associated with IL-6 therapy. This anemia has complicated immunotherapy trials and hindered the development of IL-6 as a hematopoietic growth factor. For example, Schrezenmeier et al\textsuperscript{15} were forced to halt studies of rhIL-6 in patients with aplastic anemia because of an increase in transfusion requirements, and several investigators noted enhanced anemia and transfusion requirements when rhIL-6 was used in conjunction with cytotoxic chemotherapy.\textsuperscript{16-18} This anemia has been variously postulated to be caused by RBC sequestration, hemolysis, blood loss, or diminished erythropoiesis.

Our investigations using serial RBC mass and total blood volume determinations in 6 patients treated with rhIL-6 for metastatic cancer indicate that the anemia is largely due to hemodilution secondary to a significant increase in plasma volume. A small decrease in RBC mass, at least partially related to phlebotomy, was also observed, but was not statistically significant. This increase in plasma volume is inconsistent with an RBC sequestration mechanism. Furthermore, there was no evidence to support concomitant hemolysis or gastrointestinal blood loss. The changes in serum iron, ferritin, and erythropoietin levels reported by others are unlikely to be responsible for such a rapidly developing anemia and are likely a consequence of the acute-phase response triggered by rhIL-6. Whether this mechanism applies to the anemia associated with more protracted lower-dose rhIL-6 administration remains to be determined.

A similar mechanism has been invoked for the anemia associated with IL-11 administration\textsuperscript{25} and may also contribute to the anemia seen with IL-2 therapy.\textsuperscript{26} The mechanism by which IL-6, and presumably these other cytokines, causes an increase in plasma volume is not known. Increases in serum cortisol levels, declines in myocardial contractility, or peripheral vascular dilatation related to decreases in peripheral vascular resistance may contribute. The fact that IL-6 and IL-11 share a common signal transducing molecule (gp130) may implicate this molecule in this process.

vascular leak syndrome that is routinely associated with IL-2 administration is unlikely to be a major contributor because significant vascular leak has not been reported with rhIL-6 therapy.\textsuperscript{11-12} Furthermore, IL-4 administration, which causes a profound vascular leak, has actually been associated with a rapid and transient increase in hematocrit levels, which has been presumed to be related to hemoconcentration.\textsuperscript{27} In any event, a better understanding of the mechanisms underlying cytokine-associated anemia may avoid potentially unnecessary transfusions and allow for more enlightened clinical drug development.

ACKNOWLEDGMENT

We are indebted to the NEMC Biologic Therapy Unit nurses and house officers and Hematology/Oncology Division Fellows for providing expert care of these patients, to Lucie Ronayne, RRA, for assistance in compiling the clinical data related to these studies, and to Jacqueline L. Myers for assistance in preparing the manuscript.

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