Hematologic Effects of Immunotherapy With Lymphokine-Activated Killer Cells and Recombinant Interleukin-2 in Cancer Patients

By Stephen E. Ettinghausen, Jeffrey G. Moore, Donald E. White, Leonidas Platanias, Neal S. Young, and Steven A. Rosenberg

Immunotherapy with interleukin-2 (IL-2) and lymphokine-activated killer (LAK) cells generated from autologous lymphocytes has produced significant tumor regressions in patients with advanced cancer. In the current study, we reviewed the hematologic effects associated with this therapy in our initial 42 patients. Eighty-eight percent of the treated patients developed anemia that required ≤4 units of red cell transfusions, and 43% received at least 8 units. Only a blood loss of 2 to 3 units could be attributed to repeated phlebotomy, cytopeneses, and hemodilution. IL-2 administration also resulted in thrombocytopenia as well as lymphopenia and eosinophilia. Forty-three percent of patients developed platelet counts of ≤50,000/µL, and 36% of the total group required platelet transfusions. Mild clinical summaries of our initial 42 patients undergoing their first round of treatment. Serial studies of circulating hematopoietic progenitors were conducted on four patients receiving LAK cells and IL-2 and one patient undergoing therapy with IL-2 alone. The recombinant IL-2 used in this trial was kindly supplied by Cetus Corp (Emeryville, CA). The molecular biologic and immunologic properties of this lymphokine preparation have been described elsewhere. Summaries of the clinical histories (Table I) and of hematopoietic colony assay results (Table II) are presented for the five patients who underwent analysis of the IL-2 effect upon peripheral blood progenitor colonies.

All patients had advanced cancer and had failed effective standard therapy for their specific neoplasm. Prior to the initiation of treatment, none of the patients had significant, nonneoplastic hematologic, cardiopulmonary, renal, or hepatic disease. No patient had received any therapy for at least 4 weeks before entry into this treatment trial. Informed consent was obtained from all patients before beginning therapy. The experimental immunotherapy protocol was previously approved by the Clinical Research Committee of the National Cancer Institute and by the Food and Drug Administration. The regimen for leukophereses and lymphocyte harvest and culture has been previously described in detail. Briefly, repeated lymphocytaphereses using a continuous-flow, cell separator (IBM-2997; Cope Labs, Lakewood, CO) were performed to collect 5 × 10^6 to 3 × 10^9 mononuclear cells per procedure. Lymphocytes were separated by centrifugation over Ficoll-Hypaque density gradients (LSM; Litton Bionetics, Rockville, MD) and were then placed in human AB serum-containing medium. After incubation at 37°C in roller bottles for three to four days, the LAK cells were harvested by centrifugation and were resuspended in 0.9% saline containing human serum albumin and IL-2 (375 U/mL). The cell suspension was filtered through sterile nylon mesh before infusion.

The treatment protocol has been detailed previously. Briefly, patients received three to four days of 30,000 or 100,000 U/kg of IL-2 administered as bolus intravenous (IV) infusions every eight hours. After a two- to three-day period not receiving IL-2, daily leukophereses were performed for the next five consecutive days. This schedule for the leukophereses was chosen to maximize lymphocyte yields by taking advantage of the lymphocytosis that follows the discontinuation of IL-2 infusion. The LAK cells were then administered by the IV route three to four days later. After the first cell infusion, 10,000 to 100,000 U/kg of IL-2 were injected IV every eight hours and continued for an average of four to five days. Discontinuation of IL-2 therapy was dictated by the development of
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Table 1. Patient Population

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Primary Malignancy</th>
<th>Sites of Metastases</th>
<th>Prior Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>F</td>
<td>Adenocarcinoma of the breast</td>
<td>Subcutaneous tissues, liver, lungs</td>
<td>Surgery, radiation (axilla, supraclavicular fossa)</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>F</td>
<td>Melanoma (back)</td>
<td>Lung, pancreas</td>
<td>Surgery</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>M</td>
<td>Alveolar rhabdomyosarcoma (thigh)</td>
<td>Lung</td>
<td>Surgery, chemotherapy (cyclophosphamide, doxorubicin, methotrexate, vincristine, VP-16, cisplatin, actinomycin D dacarbazine), radiation (thigh, lungs)</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>F</td>
<td>Adenocarcinoma of the lung</td>
<td>Mediastinal lymph nodes, lungs</td>
<td>Radiation (mediastinum)</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>M</td>
<td>Clear cell carcinoma of the kidney</td>
<td>Lungs, kidneys</td>
<td>Surgery</td>
</tr>
</tbody>
</table>

Table 2. Summary of Hematopoietic Progenitor Colonies Before (Pre), During, and After (Post) IL-2 Therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre</th>
<th>During</th>
<th>Post</th>
<th>Pre</th>
<th>During</th>
<th>Post</th>
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<td>BFU-E*</td>
<td>1</td>
<td>31</td>
<td>2</td>
<td>76</td>
<td>10</td>
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<tr>
<td></td>
<td>2</td>
<td>124</td>
<td>1</td>
<td>27</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>64</td>
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<td>22</td>
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<tr>
<td></td>
<td>4</td>
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<td>0</td>
<td>2,870</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>20</td>
<td>0</td>
<td>78</td>
<td>6</td>
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</table>

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre</th>
<th>During</th>
<th>Post</th>
<th>Pre</th>
<th>During</th>
<th>Post</th>
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</thead>
<tbody>
<tr>
<td>CFU-C*</td>
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<td>31</td>
<td>2</td>
<td>76</td>
<td>10</td>
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<td>0</td>
<td>78</td>
<td>6</td>
<td>0</td>
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*Values are colonies per milliliter of blood; SD, <15% of reported values.
†Minimum colony number during IL-2 treatment.
‡Maximum colony number after IL-2 treatment.

Toxicity. After a rest period, the cycle of leukophereses and LAK cell infusions with IL-2 was repeated in some patients. Some patients were treated with high-dose IL-2 alone and, therefore, did not undergo repeated leukophereses. The therapy was administered in two to six-day cycles of 100,000 to 300,000 U/kg of IL-2 given every eight hours.

During the intervals of IL-2 administration, patients received acetaminophen, indomethacin, ranitidine, hydroxyzine, doxepin, and meperidine.

Standard blood studies including complete blood counts, hepatic and renal function tests, and electrolyte determinations were frequently performed during the treatments.

Hematopoietic colony assays. The effect of immunotherapy on hematopoietic colony formation in vitro was measured before, during, and after administration of IL-2. Peripheral blood was collected in 50 units of preservative-free heparin (Forest Pharmaceuticals, St Louis) per milliliter of blood. Mononuclear cells were separated by sedimentation over Ficoll-Hypaque (LSM) and washed twice in Iscove’s modification of Dulbecco’s medium (GIBCO, Grand Island, NY) with 2% heat-inactivated fetal calf serum (FCS, twice in Iscove’s modification of Dulbecco’s medium (GIBCO, Grand Island, NY) with 2% heat-inactivated fetal calf serum (FCS, Flow Laboratories, McLean, VA). The cells were then resuspended in culture medium to a final concentration of 3 x 10^6 cells/mL. Culture medium contained 0.8% methylcellulose (Dow Chemicals, Midland, MI), 30% FCS, 1% bovine serum albumin (Sigma Chemical Co, St Louis), 100 U/mL penicillin-streptomycin (M.A. Bioproducts, Walkersville, MD), 2.5 U/mL of erythropoietin (Connaught Laboratories, Willowdale, Canada), and 10% phytohemagglutinin (GIBCO)-stimulated leukocyte-conditioned media. Cultures were plated in duplicate in Lux suspension culture dishes (35 x 10 mm; GIBCO)-stimulated leukocyte-conditioned media. Cultures were incubated in culture medium at 37°C in 95% humidity and 5% CO2. Colonies derived from primitive erythroid (BFU-E) and granulocyte/macrophage (CFU-C) progenitors were enumerated by their characteristic morphologies on day 18. The data are expressed as the number (mean ± SD) of colonies per milliliter of blood to correct for both changing total blood counts and variable recovery of mononuclear cells during treatment.

Interferon assay. γ-Interferon levels were determined with a commercial radioimmunoassay kit (Centocor, Philadelphia) using a National Institutes of Health γ-interferon standard (Gg 23-901-530) as an internal control.

RESULTS

The hematologic profiles for the initial 42 patients receiving LAK cells and IL-2 are presented in Table 3. Figure 1
Leukopenia was associated with the lymphopenic episodes of IL-2 therapy. Such as urticaria or bronchospasm were associated with the treatment or during the five days after completion of therapy. The median peak counts, 10,094 and 7,090/μL, respectively.

Discontinuation of IL-2 infusions resulted in moderate to severe thrombocytopenia (mean and median nadir counts, 67,620 and 55,000/μL, respectively), with 64% of the patients having platelet counts ≤80,000/μL and 21% having counts ≤20,000/μL. One patient whose platelet count fell as low as 20,000/μL developed an upper gastrointestinal hemorrhage, but no other significant bleeding episodes occurred as a result of thrombocytopenia.

The administration of IL-2 led to a marked lymphopenia (mean and median nadir counts, 140 and 50/μL, respectively), with lymphocyte counts falling below 1,000/μL in 98% of patients and to 0 in 45% of the individuals. After discontinuation of IL-2 therapy, a rebound lymphocytosis, which has been reported previously, was observed (mean and median peak counts, 10,094 and 7,090/μL, respectively).

Eosinophilia (mean and median peak counts, 5,913 and 2,963/μL, respectively) also developed during the course of immunotherapy and was usually observed at the end of treatment or during the five days after completion of therapy. Sixty-seven percent of the individuals had peak eosinophil counts ≥2,000/μL, whereas 38% demonstrated counts ≥5,000/μL. Although IL-2 did induce generalized fluid retention and a pruritic dermatitis, no allergic phenomena such as urticaria or bronchospasm were associated with the eosinophilia of IL-2 therapy.

Moderate leukopenia (mean and median nadir counts, 3,280 and 3,150/μL, respectively) and, in some patients, mild neutropenia (mean and median nadir counts, 2,640 and 2,490/μL, respectively) were noted during therapy. In general, leukopenia was associated with the lymphopenic episodes that occurred during IL-2 infusion and not with neutropenia.

Only three patients developed neutrophil counts of ≤1,000/μL, and none experienced septic complications.

The severity of the hematologic toxicity was reflected by extensive transfusion requirements (Fig 1). Forty-one of the 42 patients required transfusions of packed red cells, platelets, or both during therapy. Although no patient had received any other cancer treatment within 30 days of beginning immunotherapy, 14 patients had at some prior date received chemotherapy or immunotherapy, five individuals had previously undergone radiation treatments, and seven patients had received both. Red cell transfusions were administered during therapy to maintain the hemoglobin at ≥10 g/dL. Transfusions of 4 units of packed red cells were necessary in 37 patients (88%) and 8 or more units in 18 individuals (43%). Platelet transfusions were administered for bleeding in the setting of thrombocytopenia or for prophylaxis when platelet counts fell below 20,000/μL. Although 64% of patients never required platelet transfusions, eight individuals (19%) received over 20 units. One patient did not require any transfusions, whereas another patient, who suffered a gastrointestinal bleed (see the second paragraph of Results) during therapy-induced thrombocytopenia, required 19 units of packed red cells and 48 units of platelets. Except for six other patients with occult blood loss from the gastrointestinal tract and one with minor episodes of hemoptysis, there was no other evidence for significant intestinal, respiratory, or urinary tract blood losses or hemorrhage. Phlebotomy during the treatment period accounted for the loss of approximately 1 to 1/2 units of blood per patient. Hemodilution due to the administration of crystalloid and colloid solutions also contributed to changes in the concentration of various blood elements. No evidence of autoimmune disease was detected in the patients during or after therapy.

The clinical courses of the following two patients are representative of our findings. Patient 1 (Fig 2) received the combination therapy with LAK cells and IL-2, whereas patient 2 (Fig 3) was treated by high-dose IL-2 alone and did not undergo multiple leukophereses and LAK cell infusions.

With the initial administration of IL-2 (30,000 to 300,000 U/kg IV every eight hours), a significant fall in the hemoglobin level and suppression of BFU-E-derived colonies were seen when IL-2 was administered (Figs 2 and 3, Table 2). In patient 1, IL-2 produced a five- to 15-fold reduction in BFU-E colony numbers below the immediate pretreatment baselines, and in patient 2 the therapy resulted in a five- to 125-fold decrease in erythroid colonies from the baseline. Since the reduction of hemoglobin and circulating erythroid progenitor levels was observed with or without concurrent LAK cell administration, this effect appeared to represent an IL-2–mediated, LAK cell–independent phenomenon. That bone marrow function was affected by IL-2 therapy could also be inferred from the drastic decrease in colony numbers and reticulocytopenia (see patient 1, Fig 2). Although the suppression by IL-2 was evident upon both BFU-E and CFU-C colonies, the effect was much more striking upon the erythroid progenitors (Table 2). In both patients, the treatment-induced anemia necessitated 6 units of red cell transfusions. Patient 2 required 37 units of platelet concentrates during periods of severe thrombocytopenia.
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and packed red cell (PRBC) transfusions are shown. LAK cell infusions were administered as shown after leukophereses on days 8 to 12 and 22 to 26, inclusively. IL-2 was given IV every eight hours at the doses indicated.

Circulating γ-interferon could be measured during periods of IL-2 administration. For example, in patient 1 (Fig 2), the γ-interferon level was 8.5 IU/mL on day 9 (after four days of IL-2 given every eight hours) and declined to 0 by four and 60 days after completing IL-2 therapy.

Fig 2. Hematologic effects of LAK cells and IL-2, patient 1. Serial measurements of γ-interferon, erythroid (BFU-E) and granulocytic/macrophage (CFU-C) progenitors (mean ± SD), hemoglobin, reticulocyte counts, total leukocytes, neutrophils, platelets and packed red cell (PRBC) transfusions are shown. LAK cell infusions were administered as shown after leukophereses on days 8 to 12 and 22 to 26, inclusively. IL-2 was given IV every eight hours at the doses indicated.

DISCUSSION

Recently, the administration of LAK cells and recombinant IL-2 to humans with advanced cancer has produced a reduction in measurable tumor in 11 of 25 patients. However, this therapeutic regimen has been accompanied by significant toxicity. We report in this study major hematologic changes in the initial 42 patients undergoing their first cycle of immunotherapy. Anemia that required red cell transfusions occurred in almost every patient treated with LAK cells and IL-2. Thrombocytopenia (platelet count ≥50,000/μL) was observed in 43% of the individuals, and 36% of the total patient group received platelet transfusions. Sixty-nine percent of those treated developed total leukocyte counts under 4,000/μL, although severe neutropenia (≤500/μL) was only observed in one individual. Other hematologic effects included lymphopenia and eosinophilia. Since large human trials with LAK cells and IL-2 are currently under way, the knowledge of these hematologic effects and an understanding of their pathophysiology assume particular importance.

The rapidity of the decline in hemoglobin and platelet levels during therapy with IL-2 could be partly attributed to phlebotomy, hemodilution, and in some patients, gastrointestinal tract losses. In addition, in those individuals receiving LAK cells and IL-2, repeated cytophereses also resulted in small blood losses, since each cytopheresis procedure generated 350 to 550 mL of a cell suspension with a hematocrit value of 3% to 5% and 1 to 9 × 10^11 platelets. However, even these processes taken collectively could not account for the severity of the anemia and the large number of transfusions required by these patients. Blunting of the normal hematopoietic response to ongoing minor blood losses appeared likely.

We investigated the possible mechanism underlying the anemia, leukopenia, and thrombocytopenia induced by IL-2 therapy by using clonal assays for hematopoietic progenitors. Serial colony numbers were determined by using clonal assays for hematopoietic progenitors. Circulating erythroid precursors rebounded dramatically above the baseline (Figs 2 and 3, Table 2). The extent of this increase appeared to be directly related to the dose of IL-2 administered. For example, in patient 1 (Fig 2), the erythroid colony number rose 38-fold above the nadir level after 30,000 U/kg of IL-2 and 64-fold after 100,000 U/kg.

Circulating γ-interferon could be measured during periods of IL-2 administration. For example, in patient 1 (Fig 2), the highest level of interferon (15.0 IU/mL on day 6) was observed with the initial injections of IL-2 at 30,000 U/kg. However, there did not appear to be a direct dose correlation between injected IL-2 and measured interferon, since the later doses of IL-2 (100,000 U/kg) did not result in higher levels of interferon. Similarly, in patient 2, interferon levels rose to 8.5 IU/mL on day 9 (after four days of IL-2 given every eight hours) and declined to 0 by four and 60 days after completing IL-2 therapy.

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numbers with the termination of IL-2 infusions. In the only patient examined by bone marrow aspirate and biopsy during IL-2 therapy, there was absence of bone marrow precursor cells coincident with the low number of hematopoietic colonies in peripheral blood.

The hematologic changes were almost certainly secondary to IL-2 administration. The three- to 125-fold decline in erythroid colony number in the circulation of treated patients occurred with the administration of IL-2 alone as well as with the combination of LAK cells and IL-2. Moreover, these falls in colony numbers preceded the period of intensive cytophereses in patients receiving both LAK cells and IL-2. It is unlikely that LAK cells produced significant lysis of hematopoietic colonies since LAK cells have not demonstrated the ability to destroy normal tissues\cite{2,12} despite their broad lytic reactivity against a wide variety of tumor types\cite{3,4}.

Both negative\cite{13,14} and positive\cite{15,16} effects of IL-2 on hematopoiesis have been reported in colony culture. Recombinant IL-2 has been shown to inhibit hematopoiesis in vitro in a dose-dependent manner, with the effect mediated by Tac+\cite{13} or OKT8+\cite{14} cells. At low concentrations\cite{15} or under special culture conditions\cite{16}, recombinant IL-2 has also demonstrated stimulatory activity upon colony growth. These discrepant results almost certainly are due to variability among clonal progenitor assay models and the complex interactions
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between IL-2 and the repertoire of immune cells that regulate hematopoiesis in vitro.

IL-2 also has profound effects upon the number of peripheral blood lymphocytes in both animals and humans; the administration of IL-2 results in a dramatic fall in all subpopulations of lymphocytes, possibly due to a redistribution into nonrecirculating compartments. Continuous infusion of IL-2 to humans leads to an eventual rebound in all T cell subsets, including cytotoxic/suppressor cells, and the appearance of cells bearing the Tac antigen, a marker of lymphocyte activation.

IL-2 can induce the proliferation of lymphocytes in vitro and in vivo as well as stimulate cultured lymphocytes to produce lymphokines with a variety of regulatory effects. The addition of IL-2 to lymphocyte cultures can result in the induction of suppressor cells. The activity of these stimulated lymphocytes and soluble factors may, in turn, be modulated by the presence of other cells and cytokines. Serum samples from patients receiving LAK cells and IL-2 contained significant quantities of γ-interferon, an expected result given the ability of IL-2 to induce γ-interferon production in stimulated cultures. The interferons are potent suppressors of hematopoietic cell proliferation in vitro. In addition, γ-interferon may mediate suppression, is synergistic with other inhibitory cytokines, as well as stimulate the activity of a wide range of suppressive cell types. The pattern of hematopoietic suppression in these treated cancer patients is similar to the constellation of findings in individuals with aplastic anemia; in both groups, low numbers of progenitor colonies are associated with high levels of IL-2 and γ-interferon and lymphocyte activation.

Although the pathogenesis of IL-2–induced depression of hematopoietic proliferation remains unclear, it appears likely that IL-2 has both stimulatory and indirect inhibitory effects upon many subpopulations of cells within the lymphoid cell repertoire. The suppression of hematopoiesis mediated by the administration of IL-2 probably represents a complex in vivo interplay of several regulatory lymphokines.

REFERENCES

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