Peripheral eosinophilia is almost invariably observed during the course of interleukin-2 (IL-2) therapy and is frequently accompanied by the development of a capillary leak syndrome characterized by edema, weight gain, and oliguria. We studied five patients with advanced malignancy treated with IL-2. Eosinophilia was not present initially but developed in all patients late in the course of therapy, with counts ranging from 2,328/mm³ to 15,958/mm³. In all patients, there was a temporal relationship between the infusion of IL-2 and the appearance of elevated plasma concentrations of IL-5, a growth factor for eosinophils. Granulocyte-macrophage colony-stimulating factor was not detectable in plasma. IL-4 and γ-interferon plasma levels were variably elevated. Plasma concentrations of major basic protein, a toxic eosinophil granule protein, began increasing before eosinophil counts increased. By the time of the third IL-2 infusion, high concentrations of major basic protein were present in all five patients (up to 5,800 ng/mL) and skin biopsies showed major basic protein deposition in the dermis. Four patients developed significant capillary leak syndrome and all of these patients showed markedly elevated major basic protein levels. The lowest peak plasma concentration of major basic protein (1,751 ng/mL) was observed in the one patient who did not develop edema and weight gain. These results suggest that IL-2 induces IL-5 leading to marked peripheral eosinophilia and extravascular eosinophil degranulation. The release of toxic eosinophil products at extravascular sites and in the circulation may contribute to the pathogenesis of the capillary leak syndrome complicating IL-2 therapy.

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PATIENTS AND METHODS

Study design. Human recombinant IL-2 (Roche Pharmaceuticals, Nutley, NJ) was administered by continuous infusion at 3 × 10⁸ U/m²/24 hours for 4 or 5 days for four courses. Five patients (Table 1) completed the 31-day course of therapy after they had given informed consent, as approved by the Mayo Institutional Review Board. Two patients were randomized to receive LAK cells in addition to IL-2 and underwent leukapheresis on days 8, 9, and 10. LAK cells, prepared as previously described, were reinfused on days 13, 14, and 15. Nonsteroidal anti-inflammatory agents and steroids were not administered. Furosemide was often administered during the second through fourth cycles. Blood samples were obtained on days 0 through 6, 13 through 25, 28 through 31, and, in certain cases, days 8, 9, 10, or 27 for analysis of eosinophil granule proteins and circulating levels of IL-4, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), and γ-interferon (γ-IFN). Skin biopsy specimens were obtained from four of the five patients from areas of erythema and/or edema. Patient 1 was biopsied before treatment and on days 3, 14, and 28. Patient 3 was biopsied on days 16 and 31. Patient 4 was biopsied on days 1, 6, and 31. Patient 5 was biopsied on day 32.

Measurement of eosinophil major basic protein (MBP) in plasma and urine. Eosinophil granule MBP was measured by a competitive binding radioimmunoassay as described previously. Localization of MBP in skin. Tissue deposition of MBP was measured by immunohistochemistry using affinity chromatography-purified polyclonal rabbit antisera, as previously described.

Cytokine assays. Cytokines were measured in patient plasma by immunoenzymometric assay. "U"-bottom polystyrene microtiters
plates were coated with a 100 μL/well volume of the appropriate capture antibody (see below) diluted in phosphate-buffered saline (PBS) for 2 hours at 37°C. All immunoreagents used were rat monoclonal antibodies (MoAbs), except where noted (all immunoreagents were developed at DNAX, Palo Alto, CA or in collaboration with the Schering-Plough Immunology Laboratory, Lyon, France). The plates were washed thrice with 0.15 mol/L NaCl, 0.05% Tween 20 using a Microwash II (Skatron Inc, Sterling, VA) automatic plate washer. Duplicate plasma samples diluted 1:1 with an equal volume of RPMI medium containing 15% fetal calf serum (FCS) were introduced in a 50 μL/well volume for IL-5 immunoassay. For IL-4 and γ-IFN, the plasma samples were concentrated twofold, and then assayed undiluted (2X), and diluted 1:2 (1X) and 1:4 (0.5X). For GM-CSF determination, the samples were assayed undiluted. After incubation for 2 hours at room temperature and washing (as above), the appropriate nitroiodophenyl (NIP)-derivatized detecting MoAb was added in a final assay volume of 75 μL/well in 0.01 mol/L PBS containing 1 mg/mL bovine serum albumin (BSA) and 0.05% Tween 20, and incubated for 1 hour at room temperature. After washing, bound NIP-MoAb was detected by incubating 75 μL/well of J4 rat monoclonal anti-NIP immunoperoxidase conjugate (800 ng/mL) in PBS-BSA-Tween for 1 hour at room temperature. Finally, after washing, the plates were read using the VMAX microtiter plate reader (Molecular Devices, Menlo Park, CA) at 405 nm and A,650 nm. Unknown values were interpolated from standard curves prepared using recombinant standards were used in the cytokine immunoenzymetric assays performed in this study: for IL-5, JES1-39D10 (10 μg/mL) and JES1-5A10-NIP (0.5 μg/mL), with L-cell derived recombinant standard; for IL-4, rabbit IgG anti–IL-4 (10 μg/mL) and MP4-25D2-NIP (0.5 μg/mL), with CHO-derived recombinant standard; for γ-IFN, mouse MoAbs 3-6 (25 μg/mL) and B27-NIP (1 μg/mL), with Escherichia coli derived recombinant standard; and for GM-CSF, BVD2-23B6 (10 μg/mL) and BVD2-21C11-NIP (0.5 μg/mL), with E coli derived recombinant standard.

Microbioassay for IL-5. IL-5 in one patient’s plasma (patient 5) was also quantitated by a microbioassay based on mature eosinophil survival. Eosinophils were purified from heparinized peripheral blood of normal donors on a discontinuous Percoll gradient.14 Eosinophils were greater than 80% pure and greater than 98% viable. Two hundred microliter aliquots of purified eosinophil preparations (5.0 × 10⁶ cells/mL) were cultured with plasma samples or dilutions of recombinant human IL-5 (rhIL-5) in 96-well flat-bottom plates. After 4 days, cell recovery and viability were determined by staining cells with fluorescein diacetate and propidium iodide. In subsequent studies, a comparison was made of the eosinophil survival response to serial twofold dilutions of patient plasma samples and rhIL-5 preparations. To quantitate the concentration of IL-5 in patient plasma, eosinophils were cultured with 10% (vol/vol) plasma sample or rhIL-5 standard. Amount of IL-5 in samples was determined by interpolation from the standard curve.

Blocking of bioactivity in plasma by neutralizing antibody. In preliminary experiments, we found that 10 μg/mL of rat MoAb to rhIL-5, rhIL-3, or rh-GM-CSF was sufficient to abrogate eosinophil survival responses to these cytokines. The specificity of the microbioassay for IL-5 was tested by incubating plasma obtained from patient 5 with 10 μg/mL MoAb to IL-5, IL-3, and GM-CSF before culturing the plasma with eosinophils in the microbioassay.

RESULTS

Toxicity. All patients experienced fever, chills, and myalgias and these symptoms were most pronounced in patients 1, 4, and 5. Four of five patients had significant weight gain (over 7 kg during the first course of IL-2 in two patients, Table 1) and hypoalbuminemia. Periods of oliguria and, in three patients, slight elevations in serum creatinine were also noted. Patient 4 had a single episode of marked hypotension with systolic blood pressures of 60 mm Hg and transient azotemia during the second course of IL-2. With reduction of the IL-2 dose, the hypotension resolved and the subsequent two courses were tolerated at full dose. Evanescent, erythematous morbilliform skin eruptions were noted in all patients.

Eosinophilia and eosinophil degranulation. All patients had normal eosinophil counts at the initiation of therapy. Eosinophilia (greater than 1,000/mm³) was present in three patients by the day 13 and in all patients after the second course of IL-2, with peak counts ranging from 2,328/mm³ to 15,985/mm³ (Fig 1). Plasma MBP concentrations increased strikingly in all five patients (Fig 1), the highest peak being 5,600 ng/mL (normal value less than 600 ng/mL). Plasma MBP levels began to increase by day 4 or 5 of IL-2 therapy, before any significant increase in circulating eosinophils was noted. Urinary levels of MBP increased in conjunction with increased plasma levels in all patients (data not shown). There was no eosinophiluria or nonspecific proteinuria.

MBP was not detected by immunofluorescence in the two skin biopsies obtained at the initiation of IL-2. In contrast, skin biopsies obtained after commencing IL-2 therapy demonstrated extracellular MBP deposition in perivascular areas of the dermis along with variable numbers of infiltrating intact cells (Fig 2). The most prominent extracellular dermal MBP deposition was found in skin biopsy specimens from patient 3 at 31 days and patient 5 at 32 days.

Plasma IL-5 concentrations. Before administration of IL-2, IL-5 immunoreactivity was undetectable in the plasma of four of five patients (Fig 1) by the immunoenzymometric assay. In each of these four patients, plasma levels of IL-5 increased between day 2 and 6 of the first course of IL-2 and returned to baseline before the second course of therapy. With the second cycle of IL-2, plasma concentrations of IL-5 increased more rapidly (by day 2 in all four patients) and returned to less than 100 pg/mL after completion of

<table>
<thead>
<tr>
<th>Table 1. Patient Profile</th>
<th>Patient</th>
<th>Age/Sex</th>
<th>Malignancy</th>
<th>Treatment</th>
<th>Weight Gain (kg) (1st cycle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56/M</td>
<td>Renal cell</td>
<td>IL-2/LAK</td>
<td>+7.4</td>
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<td>0</td>
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<tr>
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<td>IL-2</td>
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</tr>
<tr>
<td>4</td>
<td>64/F</td>
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<td>IL-2/LAK</td>
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<tr>
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<td>68/M</td>
<td>Renal cell</td>
<td>IL-2</td>
<td>+4.9</td>
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</tbody>
</table>
IL-2 was administered by continuous infusion (3 x 10^6 U/m^2/24 h) on days 1 through 5, 13 through 17, 21 through 24 and 28 through 31. Peripheral eosinophil counts (A), plasma MBP concentrations (B), and plasma IL-5 activity (C) are shown for all five patients. In (C), data points are not connected if separated by more than 3 days.

During the third and fourth courses of IL-2, less dramatic increases in plasma IL-5 were observed in three of four patients; the fourth patient demonstrated a striking increase in plasma IL-5 during the last cycle of IL-2, with the concentration increasing from 0 to 1,290 pg/mL after only 24 hours of continuous IL-2 infusion.

In patient 5, high plasma levels of immunoreactive IL-5 were present before therapy (Fig 1C, top panel) with minimal variation in response to IL-2 administration. Like the other patients, this patient’s eosinophil count was initially normal, so we studied his plasma further to determine if the IL-5 immunoreactivity represented biologically active IL-5. Using a bioassay to measure the effect of the patient’s plasma in promoting eosinophil survival in culture, we found a low baseline level of activity with definite increases corresponding to the infusions of IL-2 (Fig 3). Serial dilutions of the patient’s plasma and rhIL-5 resulted in dose-parallel eosinophil survival curves (Fig 4), suggesting that the eosinophil viability factor in this patient’s plasma was biologically active IL-5. To further support this, blocking experiments were performed. As shown in Fig 5, the patient’s plasma (obtained on day 6 of IL-2 therapy) sustained 70% eosinophil viability after 4 days of culture as compared with approximately 20% eosinophil viability with plasma obtained at the initiation of therapy. The sustained survival was abrogated with anti-IL-5, but not with anti-IL-3 or anti-GM-CSF.

Other cytokine activity. Two other T-cell products, IL-4 and γ-IFN, were measured in plasma from these patients. In contrast to the increases in IL-5 after IL-2 administration, no specific pattern was observed with IL-4 or γ-IFN. Patient 1 had minimally detectable levels (<1 ng/mL) of γ-IFN; plasma IL-4 levels increased mid-way through therapy approaching 100 pg/mL at day 20 before quickly becoming undetectable. Patient 3 showed elevated γ-IFN at all times tested (range, 9 to 230 ng/mL), but only low levels of plasma IL-4 (32 pg/mL) for 2 days at the start of treatment. Patient 4 exhibited detectable plasma γ-IFN throughout the entire course of therapy (range, 9 to 38 ng/mL) and plasma IL-4 was detectable from day 17 onward (range, 9 to 76 pg/mL). Patients 2 and 5 had minimally detectable levels of γ-IFN at certain times, but no plasma IL-4 was detected during their courses of therapy. No patient had detectable plasma GM-CSF at anytime.

DISCUSSION

Administration of IL-2 as experimental cancer therapy is consistently associated with peripheral blood eosinophilia; however, the mechanism by which IL-2 induces eosinophilia remains to be clarified. IL-2 is an autocrine growth factor with a central role in T-cell development and immune function.15 While IL-2 itself does not induce nor enhance eosinophil differentiation,16 a growing body of evidence supports the role of T cells in this process.17-19 Three T-cell-derived growth factors are capable of maintaining eosinophil colony growth in vitro: IL-3,20 GM-CSF,21-22 and IL-5.17,23,24

Recent reports have indirectly demonstrated the presence of IL-5 (and its putative relationship to IL-2) in the plasma of rodents and humans with eosinophilia. In mice, administration of IL-2 also induces peripheral eosinophilia.21 Increased expression of messenger RNA (mRNA) for IL-5 was demonstrated in the splenocytes of these animals and the eosinophilia was suppressed by pretreatment with antibody to mouse IL-5. Antibody to IL-5 has also been shown to inhibit eosinophilia in helminth-infected mice.22 In humans, IL-5 activity has been found in the sera of three patients with idiopathic hypereosinophilic syndrome and this activity was neutralized by antibody to IL-5.23 Others have demonstrated the presence of IL-5 activity in conditioned media (containing IL-2) of T cells obtained from five patients with reactive eosinophilia24; IL-5 mRNA expression was detected in these patients’ T cells after 12 to 24 hours of culture in IL-2. In contrast, in normal subjects without eosinophilia, IL-2 did not induce IL-5 mRNA expression nor was IL-5 activity found in the...
IL-2 INCREASES PLASMA IL-5 AND EOSINOPHILS

Fig 2. Immunofluorescence localization of MBP. (A) Photomicrograph (original magnification × 400) of skin biopsy specimen from patient undergoing IL-2 therapy for malignant melanoma, stained by indirect immunofluorescence for eosinophil major basic protein. Several intact eosinophils are present and stain as brightly fluorescent ovals. In addition, there is extensive extracellular granular deposition of major basic protein in the perivascular areas of the dermis. Control serial section stained with normal rabbit IgG was negative. (B) Hematoxylin and eosin (H&E) counterstain of section in (A) (original magnification × 400) showing several intact eosinophils in a perivascular distribution. The extracellular deposition of major basic protein cannot be visualized on the H&E-stained section.

conditioned media of the IL-2–stimulated T cells. It was also recently shown that serum collected from five patients during IL-2 therapy was a potent stimulator of in vitro eosinophil colony formation by normal bone marrow. This activity was significantly inhibited by pretreating the serum with antimouse IL-5.26

Here, we have demonstrated the presence of IL-5 in the plasma of cancer patients treated with IL-2. We found that IL-2 administration was associated with a rapid increase in IL-5 levels in a group of patients with initially normal eosinophil counts. This finding is interesting in view of the earlier report that IL-2 treatment of T cells from normal controls failed to induce IL-5 message or IL-5 activity.26 Nonetheless, in our patients there was clearly a temporal relationship between infusions of IL-2 and increments in plasma levels of IL-5, with IL-5 immunoreactivity becoming undetectable between IL-2 infusions. We do not have an explanation for the very high baseline levels of immunoreactive IL-5 in our last patient. More extensive testing of this patient’s plasma, using a sensitive bioassay, showed little activity promoting eosinophil survival before therapy with subsequent induction of this activity after infusions of IL-2. The increases were abolished by anti–IL-5 but not by anti–IL-3 or anti–GM-CSF. These findings strongly suggest that IL-2 induces T cells to secrete biologically active IL-5. Furthermore, while all patients developed eosinophilia over the 31-day course of therapy, peak counts were noted more than 21 days after IL-2 therapy was begun. This delay is comparable with the time required for maximal eosinophil colony formation in bone marrow culture25 and, as suggested by recent work,29 likely reflects the effect of IL-5 on relatively immature eosinophil progenitors.

We also measured plasma levels of eosinophil granule
MBP during the course of therapy. MBP is one of several highly cationic eosinophil granule proteins and is the sole constituent of the electron-dense crystalloid core of the granule.\(^1\) The increase in plasma MBP levels preceded peripheral blood eosinophilia by more than a week, suggesting early eosinophil degranulation. The effects of IL-5 on mature eosinophils include induction of the hypodense phenotype, increased eosinophil cytotoxicity for the larvae of *Schistosoma mansoni*, and enhanced leukotriene C4 generation.\(^2\) Similar functional changes have been demonstrated in circulating eosinophils of patients treated with IL-2/LAK.\(^3\) Our findings raise the possibility that IL-5 promotes or facilitates early eosinophil degranulation. IL-5 was recently found to increase nonspecific release of eosinophil granule proteins and to enhance secretory IgA- and IgG-mediated degranulation. IL-2 alone, in similar in vitro experiments, demonstrated no such enhancing effects on the release of eosinophil granule proteins.\(^4\)

Eosinophil degranulation was evidenced not only by increased plasma levels of MBP but also by the eventual presence of this eosinophil granule protein in skin biopsies of our patients, in which it was deposited in the dermis in a perivascular distribution. Although only two patients consented to pretreatment biopsies that confirmed the absence of eosinophil degranulation before IL-2 administration, the absence of this protein in the skin of normal controls has been previously established.\(^5\) In vitro, MBP is toxic to endothelial cells.\(^6\) In vivo, it has been found at sites of tissue injury in certain diseases. Specifically, MBP is increased in the sputa of asthmatics\(^7\) and has been identified at sites of bronchial epithelial damage in patients dying of status asthmaticus,\(^8\) as well as in areas of endomyocardial necrosis in hyper eosinophilic disorders\(^9\) and in the skin of patients affected by atopic eczema and chronic urticaria.\(^10\)

Four patients developed a pronounced CLS and in these patients the increases in peripheral blood eosinophils and plasma MBP levels were striking. Others have argued against a role for eosinophils in the development of the IL-2-associated CLS, based on the onset of fluid retention before appearance (or in the rare absence) of peripheral eosinophilia.\(^11\) This argument should not be used to exclude a role for the eosinophil because our results show that MBP levels may not correlate with absolute eosinophil counts. Indeed, four patients demonstrated substantial weight gain by the end of the first week, before significant increases in peripheral eosinophil counts, but at a time when eosinophil degranulation (as evidenced by increasing plasma MBP concentrations) was ongoing. Patient 2 with the lowest peak concentration of MBP (1,751 ng/mL) and the least marked eosinophilia did not develop clinical CLS, although he did develop hypoalbuminemia. Gleich et al have described a syndrome of weight gain, edema, and marked eosinophilia\(^12\) termed episodic angioedema associated with eosinophilia, which is similar to the CLS seen with IL-2 administration. Serum concentrations of eosinophil MBP are strikingly elevated and extracellular deposition of MBP has been demonstrated by immunofluorescence in the patients' edematous skin. Recently, high levels of circulating IL-5 have also been demonstrated in the plasma of these patients (Butterfield et al, submitted).

Future studies should help clarify the role that cytokines play in the eosinophilia observed in various clinical settings. The association of peripheral blood eosinophilia and increased vascular permeability also merits further investigation.

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Administration of interleukin-2 (IL-2) results in increased plasma concentrations of IL-5 and eosinophilia in patients with cancer

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