The appearance of hypodense eosinophils during interleukin-2 treatment

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Based on membrane receptors, metabolic activity, and cell density, human eosinophils (EOSs) are a heterogeneous population of leukocytes. EOS heterogeneity translates into biologic significance, since low density cells can be metabolically more active and thus more capable of causing tissue injury. Efforts to identify mechanisms that lead to the development of hypodense EOSs have found that an in vitro exposure to cytokines reduces cell density and is associated with increased cell activity. Consequently, we evaluated the effect of an in vivo administration of interleukin-2 (IL-2) on the cell counts and density of circulating EOSs in six patients who received IL-2 as cancer biologic-modifier therapy. To determine the pattern of EOS density in relationship to IL-2 treatment, granulocyte suspensions were isolated from peripheral blood and then centrifuged over multiple discontinuous density Percoll gradients. During IL-2 treatment, the percentage of circulating hypodense EOSs increased significantly (p < 0.01) until nearly all (97.6 ± 1.6%) EOSs were hypodense (density <1.095 g/ml). Similarly, the absolute blood EOS counts significantly increased throughout treatment. On completion of IL-2 therapy, the EOS counts and density distribution returned to pretreatment values. In contrast, no increase in blood EOS counts was observed in similar patients receiving interferon (γ or β) therapy. Our observations support a hypothesis that IL-2, either directly or more likely, through the generation of other factors, participates in a change in EOS density that may, in turn, establish a subpopulation of cells with altered metabolic activity. (J ALLERGY CLIN IMMUNOL 1990;85:557-66.)

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Abbreviations used
EOS: Eosinophil
HE: Hypodense eosinophil
IL: Interleukin
IFN: Interferon
HBSS: Hank’s balanced salt solution
PBL: Peripheral blood lymphocytes
Cr: Chromium
NCI-BRMP: National Cancer Institute-Biological Response Modifiers program
Although eosinophilia is a feature of many allergic and parasitic diseases, the importance of this cell to the pathophysiology of disease has yet to be fully delineated. However, new data have begun to demonstrate that the EOS can cause tissue inflammation and injury through a variety of mechanisms, including release of its granule proteins and generation of products of oxidative metabolism. Moreover, our understanding of the phlogistic role of the EOSs has been expanded by knowledge that blood and tissue EOSs are a heterogeneous population of cells when they are evaluated for function, membrane receptors, and cell density. Low density or HES are a particularly important feature of heterogeneity because this subpopulation demonstrates increased metabolic activity and therefore an enhanced capacity for tissue injury. Accordingly, a greater understanding of the role of the EOSs in disease should follow elucidation of mechanisms that participate in the development of HES.

In this regard, in vitro studies have demonstrated that cytokines alter EOS density and metabolic activity. The capability to produce large quantities of cytokines (i.e., IL-2, the primary component of T cell growth factor) by recombinant DNA techniques makes it possible to investigate these factors in therapy for neoplastic diseases, such as renal cell carcinoma and malignant melanoma. Interestingly, patients who receive IL-2 therapy commonly develop eosinophilia with the total EOS count occasionally reaching 35,000/mm³. In the current study, we prospectively evaluated the effect of intravenous IL-2 therapy on the total number of circulating EOSs and their density in oncology patients. Furthermore, the effect of IL-2 treatment on EOS density was compared to concurrent changes in PBL function, as assessed by measuring ³¹Cr release in an in vitro cytotoxicity assay against Daudi lymphoma target cells.

MATERIAL AND METHODS

Patient population

Six patients, five male and one female patient, mean age, 42.7 ± 3.4 years (mean ± SEM), were to receive intravenous IL-2 as therapy for renal cell carcinoma or malignant melanoma as part of a larger study group and were randomly identified for study (Table I). These six patients had locally recurrent or metastatic malignant disease that was considered incurable with surgery; none had received previous treatment with IL-2. Baseline hematologic parameters, hematocrit, and white blood cell count were normal before treatment with IL-2. In addition, a group of eight healthy age-matched individuals (38.6 ± 3.5 years), six male and two female subjects, served as control subjects. These subjects were not administered IL-2 but were assessed for EOS counts and density distribution in a similar schedule as the IL-2-treated patients. Finally, a second patient control group (n = 24) consisted of subjects with melanoma, renal carcinoma, and other malignancies who received IFN therapy. None of the treated patients or normal control subjects had a history of asthma or hay fever. All patients and healthy donors signed consent forms approved by the University of Wisconsin Committee for the Protection of Human Subjects.

Study design

Each patient treated with IL-2 received four cycles during a 28-day period. Each treatment cycle consisted of four consecutive days of IL-2 administered as either a continuous 96-hour infusion or a combined continuous-bolus infusion, followed by 3 days of observation. Four patients were treated with 1 × 10⁸ U/m³/day, and the other two patients were treated with 3 × 10⁸ U/m³/day. Blood was obtained from each patient for lymphocyte cytotoxicity assays, EOS density distribution profiles, and determination of leukocyte counts on most of the following days: baseline (1 day before the first cycle of IL-2), just before the end of each 4-day IL-2 cycle, and 24, 48, and 72 hours after cessation of each 4-day cycle, as well as 4 weeks after completion of the 28-day IL-2 protocol (day 56). For the group of six patients, EOS density determinations were performed on a total of 47 separate blood specimens, with the number of specimens analyzed per patient ranging from three to 12. One patient (No. 6) was withdrawn from the study between the second and third cycles of IL-2 treatment because of disease progression; blood samples from this patient were drawn on only three occasions.

The additional group of patients with similar malignant disease were treated in a separate protocol with varying schedules of interferon therapy (Table II). These subjects were assessed for changes in EOS count only, and assessment was done to determine the specificity of peripheral blood eosinophilia to treatment regimens.

Reagents

Percoll (polyvinylpyrrolidone-coated silica gel) was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. HBSS was obtained from Grand Island Biological Co., Grand Island, N.Y. Recombinant human IL-2 was provided through the NCI-BRMP by the Hoffmann-LaRoche Co., (Nutley, N.J.). The NCI standard for unitage was used, and the specific activity of the IL-2 was approximately 15 × 10⁶ U/mg. IL-2 was provided in a lyophilized form and reconstituted with sterile normal saline. IFN-γ was provided through the NCI-BRMP by Biogen, Inc., Cambridge, Mass. IFN-β was provided by Triton Biosciences, Inc., Alameda, Calif., through the BRMP. The specific activities were 2 × 10⁷ U/mg of protein for IFN-γ and 2 × 10⁶ U/mg of protein for INF-β.

Preparation of multiple discontinuous density Percoll gradients for EOS separation

Percoll was mixed with 10 × HBSS to make a stock solution with a density of 1.124 gm/ml (as determined by pycnometer) and osmolality of 340 mOsm/kg H₂O (as determined by freezing-point depression). Aliquots of the stock Percoll were then adjusted to densities of 1.070,
TABLE I. Profile of IL-2-treated patients with cancer participating in study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Treatment (U/m²/day)</th>
<th>Completed treatment</th>
<th>Pre-IL-2 EOS/mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Melanoma</td>
<td>$3 \times 10^6$</td>
<td>Yes</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>continuous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Renal carcinoma</td>
<td>$1 \times 10^6$</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>combined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Renal carcinoma</td>
<td>$1 \times 10^6$</td>
<td>Yes</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>combined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Renal carcinoma</td>
<td>$1 \times 10^6$</td>
<td>Yes</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>combined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Melanoma</td>
<td>$3 \times 10^6$</td>
<td>Yes</td>
<td>480</td>
</tr>
<tr>
<td></td>
<td></td>
<td>combined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Renal carcinoma</td>
<td>$1 \times 10^6$</td>
<td>Stopped at 14 days</td>
<td>340</td>
</tr>
<tr>
<td></td>
<td></td>
<td>combined</td>
<td>because of progression</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>of disease</td>
<td></td>
</tr>
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TABLE II. Treatment protocols for IFN therapy

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Diagnosis</th>
<th>IFN</th>
<th>Concentration</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Melanoma (5)</td>
<td>β</td>
<td>$3-10 \times 10^6$ U</td>
<td>Continuous IV for days 1-5, repeat after 14 days</td>
</tr>
<tr>
<td>2</td>
<td>Renal carcinoma (2)</td>
<td>γ</td>
<td>200 μg</td>
<td>IV daily for 14 days, repeat after 28 days</td>
</tr>
<tr>
<td>3</td>
<td>Melanoma (6)</td>
<td>β</td>
<td>$3-30 \times 10^6$ U</td>
<td>IV daily for 14 days, repeat after 28 days</td>
</tr>
<tr>
<td></td>
<td>Renal carcinoma (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Melanoma (2)</td>
<td>γ</td>
<td>$300 \times 10^6$ U</td>
<td>Three times a wk for 2 wk, then low dose three times a week</td>
</tr>
<tr>
<td>4</td>
<td>Renal carcinoma (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IV, Intravenously.

1.080, 1.085, 1.090, 1.095, and 1.100 gm/ml by the addition of one × HBSS. Beginning with the 1.080 gm/ml solution, solutions of increasing density were underlayered in a 15 ml polystyrene tube (Corning Glass Works, Corning, N.Y.) with flexible, 21-gauge butterfly tubing connected to a hand-held syringe. Gradients consisted of 1.5 ml, 1.100; 2.5 ml, 1.095; 3.0 ml, 1.090; 3.0 ml, 1.085; and 2.5 ml, 1.080 gm/ml of Percoll.14.

Isolation of lymphocytes and preparation of granulocyte suspension

Venous blood was obtained from patients and control subjects in a syringe coated with preservative-free heparin (5000 U/ml). The blood was diluted with 10 ml of phosphate-buffered saline and underlayered with 15 ml of Ficoll-Hypaque in a 50 ml centrifuge tube.17 After centrifugation at 200 g for 30 minutes, the mononuclear cell layer was removed, washed with phosphate-buffered saline, and suspended in tissue-culture medium containing 10% human serum, as previously described.18 The remaining pellet of the Ficoll gradient contained the erythrocytes and granulocytes that were resuspended in HBSS without Ca²⁺ to a volume of 20 ml. Five milliliters of 4.5% dextran was added, and erythrocytes were allowed to sediment for 45 minutes. The granulocyte-rich supernatant was collected, washed twice in HBSS without Ca²⁺, and was then resuspended to a concentration of $20 \times 10^6$ cells per milliliter in 1.070 gm/ml of Percoll that contained 5% heat-inactivated newborn calf serum.

Separation of EOSs and neutrophils by Percoll density-gradient centrifugation

A 2 ml aliquot of the granulocyte suspension ($40 \times 10^6$ cells) was carefully layered on the top of the lightest layer of Percoll solution (1.080 gm/ml) in the discontinuous gradient. After centrifugation at 700 g for 20 minutes, cells were gently aspirated from each of the five density-gradient interfaces. The cells from each band were counted, and the purity of EOSs was determined by examining 200 cells on a Wright's-stained cytocentrifuge preparation. From these data, the fraction (percentage) of EOSs recovered from each density-gradient interface was determined.

In vitro incubation of EOSs with IL-2

To determine whether the cytokine had a direct effect on cell density, peripheral blood granulocytes were incubated in vitro with IL-2. Aliquots of $40 \times 10^6$ cells from the granulocyte suspension of two normal control subjects were
suspended in 2 ml of buffer (HBSS per 5% newborn calf serum) with or without IL-2 (100 U/ml). After incubation for 60 minutes at 37°C, eosinophil density distribution was measured by Percoll gradient as described above.

**Lymphocyte cytotoxicity assay**

In vitro assessment of lymphocyte function was performed with a Cr-release cytotoxicity assay described previously. Briefly, fresh lymphocytes from patients or healthy adult volunteers were suspended in tissue-culture medium containing 10% human serum. The cells were then cultured with IL-2 (200 U/ml for 1 hour) before the addition of target cells (in equal volume); this mixture results in an eventual IL-2 concentration of 100 U/ml after addition of target cells. 51Cr-labeled Daudi Burkitt’s lymphoma line target cells (American Type Culture Collection, Rockville, Md.) were added to varying concentrations of patients’ cells in round-bottom microwells. Radiolabel release into the supernatant was measured with results expressed as percent cytotoxicity of the indicated target cell. As reported previously, results are expressed as the percent cytotoxicity values interpolated to 17.7 lymphocytes per target cell, based on data collected at four separate lymphocyte-to-target cell ratios actually tested.

**Statistical analysis**

The effects of IL-2 treatment on EOS density, leukocyte counts, and lymphocyte cytotoxicity were analyzed with the paired Student’s t test. Correlation of data was made with Spearman’s rank test.

**RESULTS**

**The effect of IL-2 therapy on total EOS counts**

The absolute peripheral blood EOS counts rose consistently during treatment with IL-2 with the maximal mean value occurring at day 26 (2654 ± 549/mm³ versus 191 ± 74/mm³ at baseline; p = 0.0008; Fig. 1). Total lymphocyte counts also increased during therapy with the maximal mean value occurring at day 20 (3773 ± 739/mm³ versus 1345 ± 277/mm³ at baseline; p = 0.0009). The kinetics of increased cell counts varied for EOSs and lymphocytes. By the third and fourth IL-2 cycle, initiation of the IL-2 infusion correlated with a rapid rise in EOS numbers (p < 0.05) followed by a return to preinfusion levels (p > 0.05) when the 4-day treatment was completed. In contrast, for all IL-2 cycles, lymphocyte counts dropped significantly (p < 0.05) when IL-2 infusion was started and then increased (p < 0.05) again when treatment was stopped for the 3-day observation period. When cell levels were reassessed 4 weeks after therapy (day 56), both of these leukocyte measurements had returned to baseline levels. Although neutrophil and monocyte numbers did not significantly change, a modest increase in total leukocyte count was observed (data not presented).

Several patients underwent repeated courses of IL-2 therapy. For two of these patients, the variation in EOS counts for each course is illustrated in Fig. 2. Although the magnitude of the response differed, similar patterns were observed during both courses of
FIG. 3. Effect of IFN therapy (Table II) on peripheral blood EOS counts. A. Patients received combined IFN-γ and IFN-β treatment (n = 5). B. Mean EOS counts of patients receiving either IFN-γ (●, n = 8) or IFN-β (▲, n = 10).

The effect of IL-2 on EOS density

To determine the density profile for normal peripheral blood EOSs, granulocytes from eight control subjects were centrifuged on discontinuous density Percoll gradients; most EOSs (77.1 ± 4.3%) were recovered at the density interface between the 1.095 and 1.100 gm/ml of Percoll solutions. Based on these observations, EOSs recovered at the 1.095 to 1.100 mg/ml of Percoll interface were considered to be of normal density; EOSs recovered at lower densities, <1.095 gm/ml, were defined as HEs (Fig. 4). In contrast to healthy control subjects, patients with asthma have a significant increase in HE percent.

When baseline blood samples (pre-IL-2 treatment) were evaluated in the six patients with cancer, a high percentage of EOSs was hypodense (<1.095 gm/ml) as compared to EOSs from control subjects (57.4 ± 12.9% versus 22.9 ± 4.2%; p < 0.0005). Repeat determination of EOS density in each of the six patients during IL-2 therapy demonstrated an increase in the percent HE (87.8 ± 5.5; p = 0.03) after the first IL-2 cycle (Table III). In subsequent IL-2 cycles, nearly all peripheral blood EOSs (>95%) were hypodense. Four weeks after completion of the IL-2 therapy protocol (day 56), the percentage of peripheral blood HEs had decreased to 79.3 ± 8.6%, a number not significantly higher than baseline, 57.4 ± 12.9%; p = 0.12. Therefore, a shift to lower cell density was found during therapy; 4 weeks after completion of treatment, the EOS density distribution returned to pre-IL-2 levels. To determine whether the percentage of HEs also varied in control donors, blood samples were assessed weekly for several months for several normal subjects. There was little intrasubject variation in the percentage of HE (a 10% mean coefficient of variation) during this interval, and any observed variation occurred in the 1.095 gm/ml and 1.100 gm/ml density fractions (data not presented).

A shift of EOS density to lower density Percoll interfaces (<1.095 gm/ml) was not observed in normal control subjects.

In addition to determining the percentage of EOSs that were of low density, we evaluated the EOS density distribution pattern during IL-2 treatment. The EOS
FIG. 5. Change in EOS density distribution before, during, and after IL-2 therapy. Density values correspond to the heavier Percoll solution at the density interface (i.e., 1.095 gm/ml represents the 1.090 to 1.095 gm/ml interface). Bars represent the percentage of recovered EOSs that was present at the individual density interfaces. A-B, Two individual IL-2-treated patients. C, Mean of six patients ± SEM.

TABLE III. The mean percentage of peripheral blood HEs in relationship to IL-2 therapy*

<table>
<thead>
<tr>
<th>Day of treatment protocol</th>
<th>0 (before)</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>56 (after)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>% HE</td>
<td>57.4†</td>
<td>87.8</td>
<td>97.6</td>
<td>97.4</td>
<td>95.8</td>
<td>79.3</td>
</tr>
<tr>
<td>SEM</td>
<td>12.9</td>
<td>5.5</td>
<td>1.6</td>
<td>1.6</td>
<td>2.3</td>
<td>8.6</td>
</tr>
<tr>
<td>p Value††</td>
<td>0.03</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*The normal percentage of peripheral blood HEs is 22.9 ± 4.2.
†p < 0.0005 compared to percent of HEs of normal control subjects.
‡Compared with pre-IL-2 values.

density distribution for two individual patients is illustrated in Fig. 5, A and B. HEs from control subjects were confined to the 1.090 to 1.095 gm/ml density interface (designated as 1.095 gm/ml in Fig. 4). In contrast, HEs from these representative patients were present in all the low-density (≤1.095 gm/ml) cell bands. Commencing with IL-2 treatment, there was a significant shift in the distribution of EOSs at the various density gradient levels. We saw an increase in the percentage of EOSs recovered in the 1.085 and 1.090 gm/ml density bands and a concurrent decrease in cells at the 1.095 and 1.100 gm/ml bands. Four
weeks after IL-2 therapy, the EOS distribution was returning to pretherapy levels. Similar observations were observed when the mean of the EOS distribution for all six patients was calculated (Fig. 5, C). These observations indicate the degree to which the cell density changed and that the change was equivalent 2 and 5 weeks into IL-2 treatment as well as before and after IL-2 treatment.

Because of differences in drug-therapy protocol, sufficient quantities of blood were not available to determine HE density distribution from patients receiving IFN treatment.

**Lymphocyte cytotoxicity assay**

To minimize the likelihood for additional anemia, only a small volume of blood was drawn, and therefore, functional studies of EOSs, including EOSs of low density, were not done. However, concurrent measurements of lymphocyte cytotoxicity were used as an additional parameter of a leukocyte functional alteration by IL-2 therapy.

We have previously documented that the PBLs obtained from patients after in vivo IL-2 therapy demonstrate augmented direct destruction of the K562 target cell, as well as statistically significant increase in the in vitro destruction of Daudi target cells. These responses are dramatically augmented if IL-2 is present in vitro during the 4-hour Cr-release assay. The inclusion of IL-2 in the assay has a relatively small effect on increasing the destruction of Daudi target cells by lymphocytes from control donors or from patients before IL-2 therapy, in contrast to the dramatic augmentation for patients who have received in vivo IL-2. This finding is consistent with the in vivo induction by IL-2 treatment of lymphokine-activated killer cells that retain a dependence on IL-2 for their continued destructive capabilities.

The cytotoxic activity of PBLs, isolated from the same blood specimens used to measure HE, also increased during IL-2 treatment (Fig. 6). Cells from these six patients demonstrated peak lymphocyte-mediated cytotoxicity against the Daudi target on day 28 of IL-2 treatment, 47.6% ± 10.0% versus 4.2% ± 0.4% cytotoxicity at baseline; p = 0.0001. Twenty-eight days after stopping IL-2 therapy, lymphocyte cytotoxicity had nearly returned to baseline values, 7.3% ± 2.7% versus 4.2 ± 0.4%; p = 0.263. The mean percent cytotoxicity by control lymphocytes, measured simultaneously with each assay of patient cells, was 3.4% (range, 0% to 8%; n = 33).

To determine possible relationships between the different hematologic and functional parameters, Spearman's rank-correlation coefficients were calculated for changes in percent HE and cytotoxicity and for EOS and lymphocyte counts at each week of the IL-2 protocol. No significant correlations were found between changes in any of these parameters.

**Effect of in vitro IL-2 on EOS density**

In two experiments, EOSs from normal subjects were incubated in vitro with IL-2 for 60 minutes; there was no significant change in cell density. The in vitro concentration of IL-2 was 30 U/ml, a concentration selected to approximate values achieved in vivo in our patients receiving 3 × 10^6 U/m^2/day of IL-2 by continuous infusion. Longer periods of incubation were not evaluated. Certain cytokines (granulocyte/macrophage colony-stimulating factor, IL-3, and IL-5) have been reported to increase cultured EOS survival. The cultured EOSs also underwent a decrease in cell density and an enhancement of certain functional responses. Rothenberg et al. did not, however, observe prolonged EOS survival when the cells were cultured with various concentrations of IL-2.

**DISCUSSION**

When EOSs are assessed by cell density, they comprise a heterogeneous population of leukocytes whether evaluated in samples from circulating blood or fluid from bronchoalveolar lavage. Increased numbers of low-density EOSs or HEs are found in association with asthma, allergic rhinitis, idiopathic hypereosinophilic syndromes, and eosinophilic leukemia. Compared to normodense EOSs, HEs produce greater quantities of inflammatory mediators, including leukotrienes, and have enhanced cytotoxic capabilities. Thus, HEs, as opposed to EOSs of normal density, are potentially more important effector cells in tissue inflammation.
Studies by Prin et al. suggested that an increased percentage of peripheral blood HES was associated with eosinophilia and possibly reflected this condition. However, in studies of allergic rhinitis and patients with asthma, we found increased concentrations of HES, even though eosinophilia was absent. Although eosinophilia often occurs in patients with malignancies, only one of the six patients we studied had eosinophilia before IL-2 treatment. Nevertheless, four of our six subjects had an increased percentage of circulating HES. Why these patients had increased levels of HES before IL-2 treatment, especially in view of normal numbers of peripheral blood EOSs, is not known but may relate either directly or indirectly to their underlying neoplastic disease. EOS infiltration in certain malignancies has been suggested to be a possible advantage due to eosinophilic cytotoxic function against tumors, and HES have been reported to have increased cytotoxic function.

After the first week of IL-2 therapy, there was a further increase in the percentage of HES and in the number of circulating lymphocytes; the total peripheral blood EOS count did not change (Fig. 1). When leukocyte levels were reevaluated after additional IL-2 treatment, significant increases in total EOS, HES, and lymphocytes were noted. Our observations suggest that (1) the signal(s) for the development of HE may be different from the message for eosinophils and (2) more than one such cell signal appears during the course of IL-2 therapy. Furthermore, the effects of IL-2 on EOS numbers and density were not found in patients with similar underlying malignancies receiving interferons. The specificity of this EOS response to IL-2 remains, however, unresolved.

The mechanisms governing the development of HES have yet to be clearly defined. Initially, it was suggested that HES represent immature EOSs released from bone marrow. Morphologic examinations of HES, either by light or electron microscopy, do not demonstrate characteristics of immature cells. The change in cell density has also been linked to activation of mature EOSs. Increased numbers of cell-surface receptors for IgG, IgE, and complement on HES make this possibility likely. Our observations imply that in vivo administration of IL-2 may also participate in the development of HES. Etinghausen et al. found that administration of IL-2 caused peripheral blood eosinophilia in some patients. Although we also noted that IL-2 therapy elicits eosinophilia, this hematologic change occurred only after the patients already had a significant increase in the percentage of low-density EOSs. However, since a large proportion of circulating EOSs was hypodense before IL-2 therapy, we cannot conclude that similar changes in cell density will occur in individuals with normal concentrations of this subpopulation or if the IL-2 effects on circulating eosinophils are limited to situations where increased low-density cells already exist.

Although the IL-2 therapy protocol was different from our protocol, Silverstein et al. also observed changes in peripheral blood EOSs during treatment for malignancies. After a 3-week course of treatment with IL-2 and lymphokine-activated killer cells, these investigators found only slight changes in EOS density.

These reported observations document that this relatively well-tolerated IL-2 regimen can induce antitumor responses and, moreover, that our six patients demonstrated blood count changes representative of the larger group of 23 reported patients. IL-2 is a secretory glycoprotein that is produced by stimulated T-lymphocytes and can initiate proliferation of activated T cells, induce differentiation of lymphokine-activated killer cells, and stimulate T cells to produce other lymphokines. From our preliminary in vitro experiments, the effects of IL-2 on EOS density do not appear to occur from direct action of this cytokine on circulating EOSs. Whether HES are induced by the effects of IL-2 on EOS precursors or are mediated by IL-2-induced generation of a secondary cytokine (such as granulocyte/macrophage colony-stimulating factor, IL-3, or IL-5) could not be determined. Although in vitro experiments strongly argue that IL-2 receptors exist on myeloid precursors in bone marrow, the presence of this receptor on
mature, circulating EOSs has not been confirmed. Further experiments to quantitate the in vivo release of other cytokines after IL-2 therapy and analysis of myeloid responses to IL-2 in vitro are needed to address this issue more fully.

Our observations of the hemopoietic changes in patients receiving IL-2 treatment provide new information into possible mechanisms regulating EOS density in vivo. The biologic significance associated with changes in EOS density during IL-2 treatment have not been ascertained as of yet. However, since the low-density EOS is presumed to be metabolically more active than its normodense counterpart, it may well be a major participant in immune and inflammatory responses. Because of these observations, we were very interested in evaluating EOS function. However, because of the shift in density distribution, it was not possible to obtain a sufficient number of either purified EOSs or HEs to evaluate directly their function. Furthermore, it is premature to assume that all low-density EOSs have enhanced activity because data now suggest that increased responsiveness of low-density EOSs may be determined by the function tested, the stimulus used, and the particular condition or illness of the patient. Therefore, it remains speculative whether changes in EOS cell density are involved in either the in vivo antitumor effects or the clinical toxicity associated with IL-2 treatment. Further studies will be necessary to address these particular issues.

We thank the NCI-BRMP for their interest and support of this study; D. Wondrash, K. Schell, K. Nobis, K. Zarembski, and D. Melzer for technical assistance; Dr. B. Storer for statistical consultation; Drs. P. C. Kohler and J. Sosman and RNs K. Moore and A. Bochert for clinical care and analysis of patients treated with IL-2 for this study; and Dr. J. Simmons of collection of IFN data.

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