Interleukin-2 Therapy Enhances Salicylate Oxidation by Blood Granulocytes

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These studies determined the effect of interleukin-2 (IL-2) immunotherapy on the oxidative metabolism of the blood granulocytes of eight patients with metastatic renal cancer. We quantitated the rate of the hexose monophosphate shunt activity (HMPS), hydrogen peroxide (H$_2$O$_2$) production, and salicylate oxidation of the unstimulated and phorbol myristate acetate (PMA)-stimulated granulocyte cultures before, during, and after a 5-day continuous infusion of IL-2. There was no change in the rate of HMPS activity. However, the rate of salicylate oxidation of the unstimulated and PMA-stimulated cultures of these patients was significantly increased after the therapy was complete. Overall, there was no increase in the rate of H$_2$O$_2$ production, although the PMA-stimulated cultures of three of these patients had a twofold higher production of H$_2$O$_2$ after treatment compared with the pretreatment values. The enhanced rate of salicylate oxidation by the granulocytes after treatment indicates that these cells were "stimulated" in vivo to produce a potent oxidant, which is most likely hydroxyl radical or an oxidant of comparable activity. Further, the granulocytes were primed ('activated'), since they had an augmented response to PMA. IL-2 did not stimulate the oxidative metabolism of granulocyte cultures in vitro, suggesting that the IL-2 effect in vivo is not a direct one. Our results indicate that IL-2 immunotherapy is associated with the activation of blood granulocyte oxidative metabolism and that these activated granulocytes may be related to some of the toxic side effects of IL-2 therapy such as the capillary leak syndrome. Further oxidant injury to the granulocytes may explain the reported defect in chemotaxis.

MATERIALS AND METHODS

Materials. Materials were obtained from the following sources: [carboxyl-$^{14}$C]salicylate (specific activity, 56.5) and [1-14$^C$]glucose (specific activity, 4.0), ICN Radiochemicals, Irvine CA; [*$^{14}$C]formate (specific activity, 51), Amersham, Arlington Heights, IL; sodium salicylate, Aldrich Chemical, Milwaukee, WI; sodium formate, J.T. Baker Chemical, Phillipsburg NJ; taurine, catalase (C-40, 14,000 U/mg), and PMA, Sigma Chemical, St Louis, MO; Dulbecco’s phosphate-buffered saline (DPBS) and 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES), from GIBCO, Grand Island NY; and human recombinant IL-2, and proleukin (rIL-2), Cetus, Emeryville, CA. RPMI 1640 was supplied by MA Bioproducts (Walkersville, MD). Zymosan from Sigma was opsonized in serum as previously described.

Patient information and experimental design. The blood granulocytes of eight patients with documented metastatic renal cell carcinoma undergoing immunotherapy treatment with rIL-2/lymphokine-activated killer (LAK) were studied. The protocol had prior approval by the Ohio State University Human Subjects Committee and is similar to that reported by other institutions. The patients initially received a continuous infusion of rIL-2 at a dose of 3 X 10$^6$ Cetus units/m$^2$ (18 X 10$^6$ IU/m$^2$) daily for 5 days. The blood granulocytes were studied just before infusion (day 0), the third day of the infusion, and after the completion of this treatment. Five patients were studied on day 7 of the protocol and three on day 8. The patients were not studied during the second phase of the treatment. This included lymphapheresis on days 8 through 11, followed by combined therapy with rIL-2 and LAK infusions administered on days 18 to 21. The patients were...
maintained on their regular medications during the treatment. In addition, they were treated with acetaminophen for fever. One patient was treated with indomethacin for a high fever.

Isolation of patient leukocytes. Blood was collected in EDTA. Blood cells were separated by Ficoll-Hypaque density gradient procedure. Purified lymphocyte suspensions (monocyte-depleted) for the study of LAK activity were prepared from the mononuclear cell layer as previously described. The granulocytes were further purified by the following procedure. The remaining cells were resuspended in one-third volume of Seligmann’s balanced salt solution (SBSS), and 5% EDTA/SBSS (0.3 mL per each 10 mL of SBSS used for resuspension) was added to keep the EDTA concentration comparable to that used for whole blood. The granulocytes were purified by dextran sedimentation and the Ficoll-Hypaque density gradient procedure used in previously reported work. Hypotonic lysis was used to remove contaminating red blood cells.

Cytotoxicity assay. Natural Killer (NK)-resistant Daudi cells were used in all experiments as targets. Daudi cells (2 to 10 × 10^6) were incubated with 100 µCi Na^1^C^1^CrO_4_ (New England Nuclear, Boston, MA) in 0.4 mL of Tris phosphate-buffered saline for 1 hour at 37°C. The cells were washed four times with culture medium consisting of RPMI 1640 with 1 mmol/L glutamine, 20 mmol/L Hepes buffer, and 10% pooled heat-inactivated sera. Following this, the cells were resuspended in this medium at a concentration of 10^7/mL. Effector cells were suspended to various concentrations, and 0.1 mL was added to round bottomed microtiter plates (Flow Laboratories, McLean, VA). The ^1^C^1^Cr-labeled target cells were added to all wells in 0.1 mL and the plates were centrifuged at 200g for 5 minutes. After 4 hours of incubation at 37°C, the plates were centrifuged again, and 0.1 mL of supernatant was removed from each well and counted in a gamma counter. Percent lysis was calculated from the formula: (experimental cpm – spontaneous cpm)/(total cpm – spontaneous cpm) × 100. Each variable was tested in triplicate. Data are expressed as percent lysis or lytic units (LU). One lytic unit equals the number of target cells lysed × 100 by 8 × 10^4 effector cells.

Metabolic studies. The oxidation of ^1^C^1^-formate, ^1^C^1^-glucose and ^1^C^1^-salicylic acid by granulocytes before and after stimulation with PMA was used to monitor any effects of IL-2 treatment on H_2^O_2_ production, hexose monophosphate shunt metabolism (HMPS), and hydroxyl radical formation, respectively. A continuous measurement of ^1^C^1^-CO_2_ produced from the ^1^C^1^-labeled substrates was made using the ionization chamber-electrometer apparatus previously described. Purified granulocytes were incubated at 37°C with stirring in 25 mL three-neck distilling flasks. The cells were suspended in 4 mL of DPBS with 50 mg/dL glucose, at a final concentration of 4 × 10^6 cells/mL, or in a few instances, as low as 1.5 × 10^6 cells/mL at day 7 of the study. Before the addition of the cell suspension, 1 µCi or 2 µCi of ^1^C^1^-salicylate or 5 µCi of radiolabeled formate or glucose in 0.1, 0.2, or 0.5 mL, respectively, of normal saline had been added to the flasks, and any background signal had been blown off by continuously gassing the system. For the formate incubations, the flasks also contained, before the addition of cells, 10 mmol/L unlabeled formate and 25 µg/mL catalase. The saline flask contained unlabeled substrate (0.1 mmol/L sodium salicylate). After a 1-hour incubation, 50 ng/mL of PMA was added to stimulate the cells, and the suspension was further incubated for 1 to 2 hours. The peak millivolt values were used to calculate the production of CO_2_ in nanomoles per 10^7 cells per hour from the various substrates by resting or stimulated cells. The background signals produced by the system without cells ranged from 0 to 0.2 mV. The net peak mV signals above background produced by granulocyte cultures obtained from the patients before treatment were as follows: unstimulated ^1^C^1^-glucose, 1.3 to 2.9 mV; PMA-stimulated ^1^C^1^-glucose, 57 to 159 mV; unstimulated ^1^C^1^-formate, 0.1 to 0.4 mV; PMA-stimulated ^1^C^1^-formate, 7.8 to 11.3 mV; unstimulated ^1^C^1^-salicylate, 0 to 0.17 mV; PMA-stimulated ^1^C^1^-salicylate, 2 to 10 mV.

In vitro studies of the effects of rIL-2 on granulocyte oxidative metabolism. Fresh blood anticoagulated with EDTA was obtained for each experiment from healthy volunteers who had taken no medications for at least 72 hours. Granulocytes were isolated by dextran sedimentation and Ficoll-Hypaque density gradient centrifugation, as previously described, and purified from contaminating erythrocytes by hypotonic lysis. The resulting granulocytes suspensions were greater than 95% pure. Four-milliliter suspensions of the granulocytes (3.6 × 10^6 cells/mL) were incubated under similar experimental conditions to those described above for the patient studies. The effect of concentrations of rIL-2 between 333 and 2,000 Cetus units per 10^7 cells (~1,000 to 6,000 U/mL) on the oxidation of ^1^C^1^-glucose, ^1^C^1^-formate, and ^1^C^1^-salicylate was determined before and after stimulation with either PMA or opsonized zymosan. The suspensions were preincubated for 45 minutes to achieve equilibrium before adding IL-2. After an additional 15-minute incubation, opsonized zymosan (1 mg/mL) or PMA (50 ng/mL) was added to stimulate the cells. The peak mV signal produced from these cultures was used to calculate the rate of CO_2_ production from the substrate in an identical manner to that described for the patient studies.

We also determined the effect of rIL2 on the production of hypochlorous acid (HOCI). Cell incubations were set up in three-neck distilling flasks in a fashion similar to that described for the metabolic studies. Taurine, 15 mmol/L, was added to the incubations to trap the HOCl. After a 45-minute preincubation, rIL-2 was added to the culture. PMA (50 ng/mL final concentration) or zymosan (1 mg/mL) was added and the suspension incubated for an additional 1 hour. The reactions were terminated by placing the flasks into an ice bath and by immediately adding 25 µg/mL of catalsale. Cell-free supernatants were obtained by centrifugation of the suspensions at 27,700g for 5 minutes at 4°C. Next, 0.6 mL aliquots were transferred to chilled tubes. Their ability to oxidize I^- to I_2_- was determined by reaction with 2.4 mL of a 1.25% solution of KI in 0.1 mol/L phosphate buffer (pH 7.0). Results were expressed as nanomoles of I_2_- produced per 10^7 cells.

Statistical analysis. Student’s t-test for independent and paired samples was used to analyze the data.

RESULTS

LAK activity and the clinical toxicity associated with IL-2 treatment. As previously reported, most patients develop significant systemic symptoms during a 5-day IL-2 infusion. All patients developed fever. This was associated with erythroderma in most of the patients and clinically significant hypotension in six of eight patients. Some patients developed fluid retention during the administration of intravenous fluids as treatment for their hypotension. Seven of eight patients developed evidence of impaired renal function, as indicated by an increasing serum creatinine. One patient developed significant cardiac toxicity, as indicated by arrhythmias, and two patients developed pulmonary complications, as indicated by diffuse pulmonary infiltrate in one and pulmonary edema in the other. The baseline blood counts and differentials were normal in all patients before receiving IL-2 infusion (Fig 1). On the third day of the infusion, the counts were not significantly changed. On the seventh day of the study, the majority of the patients had developed mild leukocytosis. This was
IL-2 ENHANCES SALICYLATE OXIDATION BY PMN

Fig 1. Total white blood cell count and differentials of patients during IL-2 therapy. The percentages of neutrophils (PMN, □), eosinophils (EOS, □), monocytes (MON, □), and lymphocytes (LYM, □) are indicated by the shaded area under the curves.

Fig 2. Curve indicates LAK activity of blood lymphocytes at various times during treatment with IL-2. The number of lytic units is indicated on the y-axis.

associated with a decreased percentage of neutrophils, eosinophilia, and lymphocytosis (Fig 1). Systemic LAK activity was generated in all patients; maximal LAK activity was measured on day 7 (Fig 2).

Characteristics of metabolism of blood granulocytes. Table 1 summarizes the metabolism of the blood granulocytes for these patients before, during and after treatment with IL-2. Overall, there was no significant increase in the mean HMPS activity as measured by the 14C-1-glucose oxidation of the unstimulated or PMA-stimulated granulocytes. In contrast, the mean oxidation of 14C-salicylate, by both the unstimulated and PMA-stimulated granulocytes, was significantly higher than the baseline values after treatment. Of particular interest was the enhanced oxidation of salicylate by the unstimulated cells. This effect was noted in the cells of all but one of the patients. Similarly, the PMA-stimulated oxidation of salicylate was enhanced in the granulocytes of seven of eight patients studied after treatment compared with the baseline controls. There also appeared to be some augmentation of the cultures during treatment, although the changes were not statistically significant.

The effect of IL-2 infusions on the production of H2O2 was less clear. Overall, the production of H2O2 by the PMA-stimulated cultures was not significantly increased, although the cells of three of eight patients had a twofold greater production of H2O2 at day 7 compared with the baseline and third-day values. The mean values for H2O2 production for the unstimulated cultures appears to be slightly increased at 3 days and after treatment. As mentioned, the enhanced oxidation of salicylate was greatest after the infusion was completed (days 7 and 8). At this point, five of eight patients had developed eosinophilia ranging from 5% to 20% and it is unclear whether part of the enhanced metabolism of salicylate was mediated by these cells. However, there was no direct correlation between the degree of eosinophilia and the enhancement of 14C-salicylate oxidation.

Studies of the in vitro effects of IL-2 on granulocyte oxidative metabolism. In these experiments, granulocytes isolated from normal subjects were incubated with IL-2 in vitro. The rates of HMPS activity, formate oxidation, and the salicylate oxidation of unstimulated cultures are given in Table 2.

Table 1. Rate of HMPS Activity, Salicylate Oxidation, and H2O2 Production of Unstimulated and PMA-Stimulated Granulocyte Cultures of Patients Before, During, and After a Five-Day Continuous IL-2 Infusion

<table>
<thead>
<tr>
<th></th>
<th>Day 0 (baseline)</th>
<th>During</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unstimulated</td>
<td>Stimulated</td>
<td>Unstimulated</td>
</tr>
<tr>
<td>14C-1-glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>14.4</td>
<td>617.6</td>
<td>16.7</td>
</tr>
<tr>
<td>SD</td>
<td>±4.3</td>
<td>±240.4</td>
<td>±5.8</td>
</tr>
<tr>
<td><em>P</em></td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>14C-salicylate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.06</td>
<td>4.3</td>
<td>0.17</td>
</tr>
<tr>
<td>SD</td>
<td>±0.06</td>
<td>±1.8</td>
<td>.06</td>
</tr>
<tr>
<td><em>P</em></td>
<td>-</td>
<td>-</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>14C-formate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.5</td>
<td>254.4</td>
<td>9.3</td>
</tr>
<tr>
<td>SD</td>
<td>±3.2</td>
<td>±36.7</td>
<td>±5.7</td>
</tr>
<tr>
<td><em>P</em></td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are nanomoles of substrate oxidized per 10^6 cells per hour.

*P value indicates the comparison of the 3-day and 7-day or 8-day values with the corresponding values before treatment (day 0).
The oxidase is required for the reaction (unpublished result).

Table 2. Rate of HMPS Activity, H$_2$O$_2$ Production, and Salicylate Oxidation of Unstimulated Granulocyte Cultures From Normal Subjects

<table>
<thead>
<tr>
<th></th>
<th>$^{14}$C-1-Glucose</th>
<th>$^{14}$C-Formate</th>
<th>$^{14}$C-Salicylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>7.70</td>
<td>4.84</td>
<td>0.097</td>
</tr>
<tr>
<td>SD</td>
<td>$\pm$ 1.83</td>
<td>$\pm$ -</td>
<td>$\pm$ 0.05</td>
</tr>
<tr>
<td>No.</td>
<td>5</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are given in nanomoles CO$_2$ produced per $10^7$ cells per hour. The unstimulated cultures did not produce any significant amount of HOCl using the taurine assay. IL-2 did not stimulate HOCl production or alter the rate of oxidation of any of the substrates.

There was no direct effect of IL-2 on the HMPS activity, H$_2$O$_2$ production, or salicylate oxidation of these cultures (data not shown). Also, IL-2 did not stimulate the production of HOCl by these cultures. The effect of IL-2 on the metabolic burst of these cultures is given in Table 3. As shown, IL-2 did not significantly alter the metabolic burst to PMA or zymosan. These experiments also demonstrate that the oxidative metabolism of the granulocytes obtained from the patients before treatment (baseline values) is comparable to that of normal granulocytes (comparison of Table 1 with Tables 2 and 3).

**DISCUSSION**

Our results indicate that the blood granulocytes of patients receiving IL-2 infusions as immunotherapy have enhanced oxidative metabolism. This is indicated by an increased capacity of the unstimulated and PMA-stimulated cultures of these cells to metabolize salicylate after treatment with IL-2. The effect appears somewhat selective, since the enhanced salicylate oxidation was not associated with an enhanced HMPS metabolism or H$_2$O$_2$ production in the majority of cases.

We have previously characterized the metabolism of salicylate by granulocytes. This drug is decarboxylated and hydroxylated (oxidized) by a potent oxidant (ROS) produced by zymosan and PMA-stimulated granulocytes. Similar to benzoic acid, salicylate is not oxidized by the stimulated granulocytes of patients with chronic granulomatous disease. This observation indicates that NADPH oxidase is required for the reaction (unpublished result). The ROS requires superoxide (O$_2^-$) for its production and is most likely hydroxyl radical (OH) or an oxidant of comparable reactivity.

The enhanced capacity of the granulocyte cultures to oxidize salicylate without the addition of PMA after the patient received IL-2 therapy is of considerable interest, since it suggests that these cells have been stimulated in vivo by some mechanism to produce a potent oxidant. Further, our data indicate that the cells are "primed" (activated) in that they have an increased capacity to produce this ROS following stimulation by PMA. A recent report of Klempner et al also suggests that the blood granulocytes of patients receiving IL-2 immunotherapy may have activated oxidative metabolism. These investigators reported a twofold increase in the spontaneous production of superoxide following IL-2 infusion. However, an augmentation of O$_2^-$ production in cultures stimulated with FMLP or PMA was not found after the first treatment of IL-2 and only with FMLP following the second treatment (IL-2/LAK cells). In contrast, Jablons et al, using an indirect assay, reported that blood granulocytes produced less O$_2^-$ with PMA stimulation following IL-2 immunotherapy. However, our results appear to establish that the blood granulocytes of patients receiving IL-2 immunotherapy are activated. While we did not study the patients during the second phase of their treatment (IL-2/LAK infusions), it seems likely that the changes would have been more striking during this part of the treatment.

It is possible that this activation may be due to a direct effect of IL-2 on granulocyte function in vivo. However, it seems more likely that it occurs by another mechanism. First, we were unable to demonstrate any significant effect of rIL-2 on the oxidative metabolism of granulocytes in cultures supplemented with this lymphokine. Our results are similar to a few other reports. However, there is one study by Kowanko and Ferrante which suggests that IL-2 may directly activate the oxidative metabolism of granulocyte cultures in vitro. The effect was more striking with purified IL-2 prepared from mitogen-stimulated leukocytes than with rIL-2. Our negative results compared with those of Kowanko and Ferrante could relate to the differences in our culture conditions. Therefore, a direct effect of IL-2 on the oxidative metabolism of granulocytes in vivo cannot be excluded completely. However, the granulocyte cultures

Table 3. Effect of IL-2 on the Metabolic Burst of Normal Granulocyte Cultures to PMA and Zymosan

<table>
<thead>
<tr>
<th></th>
<th>$^{14}$C-1-Glucose</th>
<th>$^{14}$C-Formate</th>
<th>$^{14}$C-Salicylate</th>
<th>$I_2$ Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMA Control</td>
<td>921.9 ± 101 (3)</td>
<td>381.3 (2)</td>
<td>4.17 ± 1.01 (5)</td>
<td>118.6 ± 17.1 (3)</td>
</tr>
<tr>
<td>+333 U IL-2*</td>
<td>731.2 ± 61 (3)</td>
<td>359.5 (2)</td>
<td>3.86 ± 1.77 (3)</td>
<td>117.4 ± 8.7 (3)</td>
</tr>
<tr>
<td>+1,000 U IL-2</td>
<td>ND</td>
<td>ND</td>
<td>4.47 ± 0.78 (3)</td>
<td>107.1 ± 34.6 (3)</td>
</tr>
<tr>
<td>+2,000 U IL-2</td>
<td>845.9 ± 19 (3)</td>
<td>401.1 (2)</td>
<td>3.81 ± 1.61 (4)</td>
<td>122.0 ± 20.0 (3)</td>
</tr>
<tr>
<td>Zymosan Control</td>
<td>381.2 (2)</td>
<td>ND</td>
<td>7.53 ± 1.91 (3)</td>
<td>216.8 (2)</td>
</tr>
<tr>
<td>+333 U IL-2</td>
<td>325.5 (2)</td>
<td>ND</td>
<td>7.30 ± 2.30 (3)</td>
<td>217.8 (2)</td>
</tr>
<tr>
<td>+1,000 U IL-2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>233.1 (2)</td>
</tr>
<tr>
<td>+2,000 U IL-2</td>
<td>377.2 (2)</td>
<td>ND</td>
<td>8.42 ± 2.29 (3)</td>
<td>226.9 (2)</td>
</tr>
</tbody>
</table>

Concentrations of IL-2 are given in Cetus units per $10^6$ cells. The rates of oxidation of $^{14}$C-1-glucose, $^{14}$C-formate, and $^{14}$C-salicylate ± SD are given in nanomoles per $10^7$ cells per hour. The number of experiments is indicated in parentheses. The HOCl produced was estimated by the nanomoles of $^{14}$C oxidized to $I_2$. The value indicates the amount of $I_2$ produced per $10^7$ cells. ND, not done.
obtained after the IL-2 infusion was complete were more activated than those obtained during the IL-2 infusion. Therefore, it seems likely that the effect is mediated by another stimulus induced in vivo by the IL-2. IL-2 infusions have been shown to be associated with increased serum concentrations of tumor necrosis factor, interferon, IL-1, and granulocyte colony-stimulating factors. Some of these cytokines have been reported to directly activate phagocytic cells in vitro and in vivo. Therefore, it is possible that one of these cytokines may be the mediator of the effect. Alternately, the changes could be secondary to an activation of serum complement either by a cytokine or by another as yet undetermined mechanism.

Our observation that the blood granulocytes of patients receiving IL-2 infusions have activated oxidative metabolism may relate to some of the clinical problems occurring in these patients. First, the activation of the granulocytes may relate to the development of the capillary leak syndrome. In this regard, it has been postulated that the release of toxic compounds by activated phagocytic cells, particularly ROS, may mediate the vascular injury occurring in a number of clinical disorders. This vascular injury could then be mediated by activated granulocytes following their adherence to endothelium, particularly if the increased ROS released from these cells is associated with a simultaneous release of prostaglandins and enzymes. If the release of a potent oxidant by granulocytes is related to the capillary leak syndrome occurring in patients receiving IL-2 immunotherapy, then the administration of an antioxidant drug might reduce or eliminate the symptoms related to this complication. Also, our results confirm reports which indicate that IL-2 immunotherapy is associated with eosinophilia. As indicated in Fig 1, this was noted after completing the first phase of the treatment and increased further during the second part of the treatment with IL-2-LAK. This observation suggests that eosinophils, as well as neutrophils, may mediate some of the vascular injury occurring in patients during IL-2 immunotherapy. Further, recent studies indicate that other lymphokines (IL-3 or IL-5) may stimulate the production of eosinophils. This suggests that IL-2 immunotherapy may also be associated with an increased production of these cytokines.

Other observations have suggested that the increased incidence of infections (primarily catheter-related) occurring in patients receiving IL-2 immunotherapy may be secondary to an acquired defect in granulocyte chemotaxis. Our results, which indicate that these granulocytes are producing a potent oxidant, may relate directly to the reported membrane changes and impaired chemotaxis of these cells, since oxidant damage to granulocytes and other cells has been shown to impair the chemotactic response and membrane function. Klempner et al have recently reported that the blood granulocytes of patients receiving IL-2 infusions have impaired chemotaxis to both FMLP- and zymosan-activated serum. This defect in cell function does not appear to be associated with impairment of other cellular functions, since random migration, phagocytosis, bactericidal activity, and the secretion of neutrophil granules were not impaired. Jablons et al reported that the impaired chemotaxis is also associated with membrane abnormalities, since Fcy R III receptor expression was markedly decreased. Our observation that granulocytes have an augmented oxidative metabolism suggests that the impaired chemotactic function occurring in the blood granulocytes of patients following IL-2 treatment may be a secondary rather than a primary phenomenon, which results from an initial activation of their oxidative metabolism.

Additionally, a number of recent reports indicate that the release of ROS by phagocytic cells may directly impair lymphocyte function including killer activities. We have previously suggested that the release of ROS by phagocytic cells in vivo may represent a feedback mechanism for downregulation of lymphocyte function (also see Sagone). While we did not evaluate monocyte-macrophage function in this study, these cells may be activated in vivo in a similar manner to granulocytes. Therefore, the in vivo release of ROS by granulocytes or mononuclear phagocytic cells might be associated with a downregulation of LAK activity. In this regard, it is of interest that the LAK activity of the blood lymphocytes of our patients appeared to decline at the same time that we observed enhancement of granulocyte oxidative metabolism (comparison of Table 1 and Fig 2).

Finally, our results have one additional and possibly broader implication, since activated granulocytes may occur in a variety of clinical disorders. We have previously proposed that activated granulocytes may have an enhanced capacity to metabolize xenobiotics in vivo. Our finding that the blood granulocytes of patients receiving IL-2 treatment have an increased capacity to metabolize salicylate, an important antiinflammatory drug, appears to support this concept. The possibility that the activated granulocytes of patients in a variety of clinical disorders have a similar capacity requires additional study.

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