Effect of Recombinant Human Interleukin 4 on Human Monocyte Activity

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ABSTRACT

Recombinant human interleukin 4 (rhuIL-4), a lymphokine that reportedly stimulates tumoral activity in mouse macrophages, is currently undergoing clinical studies to determine its efficacy in the treatment of cancer. IL-4 is known to participate with other cytokines to regulate growth and differentiation of various hematopoietic cells as well as modulate immune response. Little is known about the effect of rhuIL-4 on human monocyte tumoral activity. The purpose of these studies was to examine the effect of rhuIL-4 on human peripheral blood monocytes. Peripheral blood monocytes isolated from normal donors failed to demonstrate tumoral activity or interleukin 1 secretion after treatment with rhuIL-4 in vitro. Furthermore, monocyte-mediated cytotoxicity induced by recombinant human gamma interferon plus muramyl dipeptide was suppressed in a dose-dependent manner by rhuIL-4. This reduction in cytotoxicity corresponded to a reduction in IL-1 production and secretion. Further investigation of rhuIL-4 and its role in the cytokine network is necessary for the development of effective immunotherapy in cancer patients.

INTRODUCTION

IL-4 is a M, 20,000 T-cell product first described as a costimulant with anti-IgM antibodies for entry of B-cells into the S phase of the cell cycle (1). It is now known to have multiple other effects as well. IL-4 enhances the colony formation of a variety of hematopoietic progenitor cells in coinulation with other specific growth factors (2, 3) and stimulates the growth of normal T-cells, certain T-cell lines, and mast cell lines (4, 5). IL-4 has also been shown to enhance the expression of class II major histocompatibility complex antigens on resting B-cells and macrophages and to markedly increase the production of IgG and IgE by B-cell populations stimulated with LPS (6–9).

Murine IL-4 has been reported to stimulate macrophage tumoral activity (10). Thus, rhuIL-4 produced by a strain of yeast containing a modified version of the human IL-4 gene is currently undergoing clinical studies to determine its efficacy in the treatment of cancer. In the present investigation, we determined the effect of rhuIL-4 on the tumoral activity of normal human monocytes in vitro.

MATERIALS AND METHODS

Reagents and Drugs. RPMI 1640, HBSS without Ca\(^{2+}\) or Mg\(^{2+}\), FBS, human AB serum, and Eagle’s CMEM were purchased from M. A.

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4 The abbreviations used are: IL-4, interleukin 4; LPS, lipopolysaccharide; rhuIL-4, recombinant human interleukin 4; HBSS, Hanks’ balanced salt solution; FBS, fetal bovine serum; CMEM, complete minimal essential medium; rhuIFN-\(\gamma\), recombinant human gamma interferon; PBM, peripheral blood monocytes; MDP, muramyl dipeptide; [\(^{125}\)I]tdUrd, [\(^{125}\)I]iododeoxyuridine; IL-1, interleukin 1; dThd, thymidine; rhuIL-1q, recombinant human interleukin 1q; rhuIL-1\(\beta\), recombinant human interleukin 1\(\beta\).

Bioproducts (Walkersville, MD). rhuIFN-\(\gamma\), supplied by Genentech, Inc. (South San Francisco, CA), had a specific activity of 2 x 10\(^4\) units/mg and a purity level of >95%. Recombinant human interleukins 1\(\alpha\) and 1\(\beta\) (specific activity, 10\(^5\) units/mg) and rabbit anti-human interleukins 1\(\alpha\) and 1\(\beta\) (polyclonal antisera) were supplied by Genzyme Corp. (Boston, MA). The rhuIL-4 was the generous gift of Sterling Drug, Inc. (Malvern, PA). The purified rhuIL-4 had a specific activity of 1.5 ± 0.4 x 10\(^4\) units/mg. All reagents were free of endotoxin as determined by the Limulus amebocyte lysate assay (sensitivity limit, 0.025 ng/ml). Salmonella typhosa LPS was purchased from Sigma (St. Louis, MO).

Separation of Normal Donor Monocytes. Mononuclear leukocytes from the peripheral blood of normal blood donors were collected on lymphocyte separation medium (Litton Bionetics, Kensington, MD) by centrifugation at 1300 x g for 10 min. The PBM were then centrifuged twice in HBSS (500 x g for 15 min) to remove platelets and suspended in RPMI 1640 supplemented with 5% human AB serum. The percentage of monocytes in the mononuclear layer was assessed by morphology and peroxidase staining, and the cell suspension was adjusted to contain 10\(^5\) monocytes/ml. Monocytes (2 x 10\(^5\)) were added to each well of a 96-well flat-bottomed Microtest II plate (Falcon Plastics, Oxnard, CA) and allowed to adhere for 1.5–2 h at 37°C. After incubation, the nonadherent cells were removed by washing three times with HBSS. The plating efficiency of the monocyte monolayers was 90%. The monocyte purity was >97% as assessed by India ink ingestion, morphology, and peroxidase staining.

In Vitro Activation of Human Monocytes. The monocyte cultures were preincubated with rhuIL-4 (0.5–100 ng/ml) for 1 h before activators (RPMI 1640 containing 5% human AB serum and LPS (1 mg/ml) or rhuIFN-\(\gamma\) (10\(^4\) units/ml) plus MDP (100 ng/ml)) were added. After an incubation period of 18–24 h, the culture wells were washed twice with HBSS to remove the rhuIL-4 and the activating agents and then [\(^{125}\)I]tdUrd-radiolabeled target cells were added as described below.

A second group of monocyte cultures was incubated with the activating agents (LPS or rhuIFN-\(\gamma\) plus MDP) for 18–24 h. The culture wells were then washed twice with HBSS to remove the activating agents, and rhuIL-4 (0.5–100 ng/ml) and [\(^{125}\)I]tdUrd-radiolabeled target cells were added.

Target Cell Cultures. A375, a cultured cell line derived from human melanoma, was provided by Dr. Raymond Rudder (National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD). Monolayer cultures were maintained on plastic in CMEM supplemented with 10% FBS, sodium pyruvate, nonessential amino acids, 2-fold-concentrated vitamin solution, and L-glutamine (M. A. Bioproducts) at 37°C in an incubator providing a humidified atmosphere containing 5% CO\(_2\). All cultures were free of Mycoplasma and pathogenic murine viruses.

Assay of Monocyte-mediated Cytotoxicity. Cytotoxicity was assessed by lysis of radiolabeled A375 tumor cells in a 72-h assay. Target cells in growth phase were incubated for 24 h in medium containing [\(^{125}\)I]tdUrd (0.3 μCi/ml; specific activity, 200 mCi/μmol; New England Nuclear, Boston, MA). The cells were washed twice to remove unincorporated radiolabel and harvested after trypsinization using 0.25% trypsin (Difco, Detroit, MI) and 0.02% EDTA for 1 min and washed again.

The labeled cells were resuspended in CMEM supplemented with 5% FBS, and 10\(^5\) cells were plated into the culture wells to provide a 1:20 target:effector cell ratio. Radioiodinated tumor cells were also plated alone as an additional control group. The culture wells were washed after 24 h to remove nonadherent tumor cells, refed with fresh medium, and then incubated for an additional 48 h. The cultures were then washed twice with HBSS, and the adherent viable cells were lysed with 0.1 ml of 0.5 N NaOH. The radioactivity of the lysate was measured in a gamma counter. The percentage of tumor cells killed by monocyte-
mediated cytosis was calculated by two different formulas. In the first, we calculated the percentage of cells lysed by monocytes collected from cancer patients without further in vitro treatment:

Cytotoxicity (%) = \( \frac{A - B}{A} \times 100 \)

where \( A \) is the cpm for target cells cultured alone, and \( B \) is the cpm for target cells cultured with monocytes. We previously determined that natural killer cell activity does not contribute to the cytotoxicity value (11) and that this assay measures lysis and not target cell detachments (12).

The second formula, which measured the effects of incubating monocytes with LPS, rhuIFN-γ plus MDP or rhuIL-4 in vitro, calculated the percentage of tumor cells lysed by the formula

\[
\text{Generated cytotoxicity} = \left( \frac{A - B}{A} \right) \times 100
\]

where \( A \) is the cpm for target cells cultured with untreated monocytes, and \( B \) is the cpm for target cells cultured with in vitro-treated monocytes.

Statistical Analysis. Experimental results were analyzed for their statistical significance by Student’s t test.

Preparation of Supernatants for IL-1 Assays. To determine whether rhuIL-4 stimulates monocyte production of soluble IL-1, PBM obtained from normal donors were separated as described above. Monocytes (10⁶) were added to each well of a 24-well flat-bottomed plate (Falcon Plastics) and allowed to adhere for 1.5-2 h at 37°C. Nonadherent cells were then removed by three washes with HBSS. rhuIL-4 (100 ng/ml) or LPS (1 µg/ml) or rhuIFN-γ (10⁴ U/ml plus MDP (100 ng/ml) was added to the culture wells. A second group of monocytes was treated with LPS (1 µg/ml) or rhuIFN-γ (10⁴ units/ml plus MDP (100 ng/ml) in the presence of rhuIL-4 to determine whether rhuIL-4 had any effect on the abilities of these activators to stimulate monocyte-mediated IL-1 production. Supernatants were collected separately 24 h later, spun at 225 x g for 5 min to remove residual cells and cellular debris, and stored at 20°C. The monocyte cultures were then reseeded with RPMI 1640 supplemented with 5% human AB serum, and the supernatants were again collected 24 h later (48 h after the initial stimulation), spun, and frozen.

For preparation of intracellular IL-1, the monocyte monolayers were washed thoroughly, fed with fresh medium, frozen at -20°C, and thawed. The freeze-thawing process was repeated three times, after which the preparations were centrifuged and the supernatants were harvested.

D10.G4.1 Assay. The murine helper T-cell clone D10.G4.1, kindly provided by Dr. C. Janeway (Yale School of Medicine, New Haven, CT), was used to measure intracellular and extracellular IL-1 activity as described previously (13). Following exposure to IL-1 and concanavalin A, these T-cells proliferate, and their proliferation can be quantified by measuring [³H]Tdr incorporation. Briefly, D10.G4.1 cells were cultured in bulk quantities for 14-16 days following exposure to H₂O₂ antigen-presenting spleen cells (C56BL/6) and were frozen at 2.5 x 10⁴ cells/µl in medium containing 20% FBS and 8% dimethyl sulfoxide. On the day of the assay, the cells were quickly thawed, washed twice with medium, and seeded at 2 x 10⁶ cells/well in medium containing 2.5 µg/ml concanavalin A (Sigma, St. Louis, MO) and with serial dilutions of test and positive control supernatants in 96-well microtiter plates. After 48 h incubation, 0.2 µCi of [³H]Tdr was added to each well. The cells were harvested in a semiautomatic cell harvester (LKB Wallac-Skatron, Inc., Sterling, VA). The amount of [³H]Tdr incorporated was quantified using a scintillation counter.

RESULTS

Effect of rhuIL-4 on Normal Blood Monocyte Tumoricidal Activity. The PBM from 8 healthy laboratory personnel were isolated and incubated with rhuIL-4 (0.5-100 ng/ml) for 24 h and then washed prior to the addition of radio-labeled A375 melanoma cells. Monocytes from all 8 donors were activated by both LPS (mean cytotoxicity, 61.4 ± 19.3%; range, 23-85%) and rhuIFN-γ plus MDP (mean cytotoxicity, 42 ± 10.2%; range, 22-52%) to kill tumor cells. Normal donor monocytes failed to show significant cytotoxicity following incubation with rhuIL-4 (mean cytotoxicity, 1.9 ± 3.4%; range, 0-8%).

Human monocytes require both a priming and a triggering signal in order to be activated to the tumoricidal state. rhuIFN-γ can function as the priming signal and LPS or MDP can serve as the triggering signal (14, 15). Because rhuIL-4 is also a lymphokine, as tested whether it required a second signal to initiate tumoricidal activity. rhuIL-4 was therefore combined with suboptimal doses of LPS, MDP, or rhuIFN-γ during the 24-h activation phase. As shown in Table 1, rhuIL-4, when used alone or in combination with suboptimal doses of rhuIFN-γ, LPS, or MDP, again failed to stimulate the antitumor activity of normal-donor monocytes.

Effect of rhuIL-4 on Monocyte-mediated Cytotoxicity in Vitro. The first 24-h period in the cytotoxicity assay is referred to as the “activation phase.” The subsequent 72-h period, when tumor cells are cocultivated with monocytes, is referred to as the “effector phase.” To determine whether rhuIL-4 had any effect on the activation process of monocyte tumoricidal function, peripheral blood monocytes from normal donors were incubated with rhuIL-4 (0.5-100 ng/ml) for 24 h (activation phase only) in the presence of rhuIFN-γ plus MDP (10⁴ units/ml plus 100 ng/ml). Monocyte cultures were then washed, [³H]IdUrd-labeled A375 melanoma cells were added, and cytotoxicity was determined 72 h later. rhuIL-4 was not present during the effector phase. Monocytes incubated with rhuIFN-γ plus MDP in the presence of rhuIL-4 for 24 h showed a dose-dependent suppression of monocyte-mediated cytotoxicity (Fig. 1). Monocytes incubated with rhuIFN-γ plus MDP alone had cytotoxicity values of 47 ± 0.1% (SD) against the target tumor cells. The addition of ≥5 ng/ml rhuIL-4 to monocyte cultures during the activation phase significantly reduced the generated cytotoxicity (P = 0.002). rhuIL-4 did not affect cytotoxic activity when present only during the effector phase (data not shown). Thus, once the monocytes were activated, rhuIL-4 could not.

Table 1 Effect of rhuIL-4 in vitro on triggering of monocyte tumoricidal activity

<table>
<thead>
<tr>
<th>Treatment of monocytes</th>
<th>Generated cytotoxicity (%)</th>
</tr>
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<tbody>
<tr>
<td>Medium</td>
<td></td>
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<tr>
<td>LPS</td>
<td></td>
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<tr>
<td>1 µg/ml</td>
<td>76</td>
</tr>
<tr>
<td>100 ng/ml</td>
<td>63</td>
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<tr>
<td>10 ng/ml</td>
<td>47</td>
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<tr>
<td>1 ng/ml</td>
<td>8</td>
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<tr>
<td>0.1 ng/ml</td>
<td>0</td>
</tr>
<tr>
<td>0.01 ng/ml</td>
<td>0</td>
</tr>
<tr>
<td>MDP (100 ng/ml)</td>
<td>12</td>
</tr>
<tr>
<td>rhuIFN-γ (100 units/ml)</td>
<td>7</td>
</tr>
<tr>
<td>rhuIL-4</td>
<td>7</td>
</tr>
<tr>
<td>100 ng/ml</td>
<td>7</td>
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<tr>
<td>75 ng/ml</td>
<td>3</td>
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<td>25 ng/ml</td>
<td>4</td>
</tr>
<tr>
<td>8 ng/ml</td>
<td>5</td>
</tr>
<tr>
<td>5 ng/ml</td>
<td>0</td>
</tr>
<tr>
<td>0.5 ng/ml</td>
<td>0</td>
</tr>
<tr>
<td>rhuIFN-γ + MDP (10⁴ units/ml + 100 ng/ml)</td>
<td>47</td>
</tr>
<tr>
<td>rhuIFN-γ + rhuIL-4 (10⁴ units/ml + 100 ng/ml)</td>
<td>16</td>
</tr>
<tr>
<td>rhuIL-4 + MDP (100 ng/ml + 100 ng/ml)</td>
<td>10</td>
</tr>
<tr>
<td>rhuIL-4 + LPS (100 ng/ml + 1 ng/ml)</td>
<td>6</td>
</tr>
</tbody>
</table>

* Normal monocytes (2 x 10⁶) were incubated with the indicated material for 24 h and washed before the addition of 10⁴ [³H]IdUrd-labeled tumor target cells.

As compared with monocytes treated with medium. Values ≤15% are not significant in this assay.

Percentage of cytotoxicity as compared with tumor cells alone.
suppress their cytotoxic function.

**Effect of rhuIL-4 on Monocyte-mediated IL-1 Production.**

Because monocyte-mediated tumoricidal activity is closely associated with IL-1 secretion (16-18), we tested the effect of rhuIL-4 on monocyte-mediated IL-1 production. Monocyte cultures that had been incubated with rhuIL-4 for 24 h served as a control. The cultured supernatants were harvested and then assayed for the presence of IL-1. Medium-treated monocytes do not release IL-1 under these conditions (19). Monocytes incubated with LPS or rhuIFN-γ plus MDP in the absence of rhuIL-4 served as positive controls for IL-1 production. Supernatants collected at 24 and 48 h after treatment with rhuIL-4 alone had no IL-1 activity (data not shown), while positive control supernatants collected from monocytes stimulated with LPS or rhuIFN-γ plus MDP contained significant IL-1 activity (data not shown). Therefore, we concluded that rhuIL-4 does not by itself stimulate IL-1 secretion.

We next investigated whether the suppression of monocyte-mediated cytotoxicity due to rhuIL-4 (Fig. 1) was associated with a reduction in IL-1 synthesis. Monocyte cultures were incubated with rhuIFN-γ plus MDP (10^6 units/ml plus 100 ng/ml) in the presence of rhuIL-4 (100 ng/ml) to determine if rhuIL-4 interfered with monocyte-mediated IL-1 production. Supernatants obtained from monocytes cultured for 24 and 48 h with rhuIFN-γ, MDP, and rhuIL-4 had significantly reduced IL-1 activities compared with those of supernatants obtained from monocytes cultured with rhuIFN-γ plus MDP alone (Fig. 2) (24-h supernatant, P = 7.9 x 10^{-5}; 48-h supernatant, P < 10^{-6}). Cell lysates obtained from monocytes cultured with rhuIFN-γ plus MDP alone or in combination with rhuIL-4 failed to show significant IL-1 activity indicating that by 48 h, no cell-associated IL-1 could be detected in these monocytes. rhuIL-4, when added to positive control wells containing IL-1α or IL-1β (data not shown), had no effect on the proliferation of the D10.G4.1 cells, excluding the possibility that rhuIL-4 remaining in the culture fluid interfered with the IL-1-induced proliferation of the D10.G4.1 cells or nonspecifically killed them.

Monocyte cultures were also incubated with LPS in the presence of rhuIL-4 to determine the effect of rhuIL-4 on that agent which is known to activate monocyte tumoricidal properties. Monocytes incubated with LPS and rhuIL-4 also had significantly reduced IL-1 activity in the 48-h supernatant and in the cell lysate fraction as compared to monocytes incubated with LPS alone (48-h supernatant, P = 2.84 x 10^{-7}; cell lysate, P = 1.5 x 10^{-3}) (Fig. 2).

The D10.G4.1 assay used does not distinguish between IL-1α and IL-1β activity. Therefore, we used rabbit anti-human polyclonal antisera against human IL-1α and IL-1β to determine if this inhibition of IL-1 activity was selective. Monocytes were incubated with either rhuIFN-γ plus MDP (10^6 units/ml plus 100 ng/ml) or LPS (1 µg/ml) in the presence of rhuIL-4 (100 ng/ml). The 24- and 48-h supernatants and cell lysates were collected as described above and divided into 4 fractions to which the following antibodies were added: Fraction 1, no antisera; Fraction 2, rabbit anti-human IL-1α polyclonal antiserum; Fraction 3, rabbit anti-human IL-1β polyclonal antiserum; and Fraction 4, both rabbit anti-human IL-1α and IL-1β polyclonal antisera. The fractions were incubated with the antisera (5 x 10^{-6} unit antisera/0.05 ml) for 24 h at 4°C and then run in the D10.G4.1 assay to determine IL-1 activity.

In control studies, anti-IL-1α (5 x 10^{-6} unit) neutralized rhuIL-1α (50 units/ml) but not rhuIL-1β (data not shown). Similarly, anti-IL-1β (5 x 10^{-6} unit) neutralized rIL-1β (10 units/ml) but not rIL-1α. We previously determined that LPS-stimulated monocyte supernatants contain 5-10 units/ml of IL-1 activity (20). Therefore, the concentration of antibody used was sufficient to neutralize all IL-1 activity. Indeed, IL-1 activity was abolished from both the LPS and rhuIFN-γ plus MDP supernatant fractions following incubation with both anti-IL-1α and anti-IL-1β (Fig. 3). We interpret our results to mean that any IL-1 activity remaining in the supernatant treated with anti-IL-1α must therefore be due to IL-1β and that activity following treatment with anti-IL-1β is due to IL-1α.

Numerous investigators have described the pattern of IL-1 secretion by monocytes after stimulation with LPS (21-23). IL-1β is secreted continuously beginning 2 h after synthesis and has a half-life of 2.5 h. The secretion of IL-1α is delayed for 10 h, and its half-life is 15 h. Our data were consistent with these previous reports on the kinetics of IL-1 secretion. At 24 h post-stimulation by LPS, IL-1 activity in the supernatants was due to the combined activities of IL-1α and IL-1β. IL-1 activity was abolished when both anti-IL-1α and anti-IL-1β were present (Fig. 3) but not when either antibody was used alone (Fig. 3). By 48 h (please note that this represents what the cell has
secreted over the second 24-h period; see “Materials and Methods”), the IL-1 activity resulted from the secretion of only IL-1α; anti-IL-1α but not anti-IL-1β abolished all activity in the D10.G4.1 assay. The intracellular fraction (cell lysate) likewise demonstrated IL-1α activity exclusively (Fig. 3).

Supernatants obtained from monocytes incubated with LPS plus rhuIL-4 had reduced IL-1α and IL-1β activity at 24 h (Table 2). The IL-1α activity in the 48-h supernatant and cell lysate fractions from the monocytes was significantly reduced compared with that in supernatants obtained from monocytes incubated with LPS alone (Table 2). The inhibition of IL-1β activity was not significant.

The pattern of IL-1 secretion from monocytes stimulated by rhuIFN-γ plus MDP was similar to that of monocytes stimulated by LPS (Fig. 3; Table 2). Cultured supernatants from monocytes incubated with the agents for 24 h contained both IL-1α and IL-1β (Table 2), but by 48 h, only IL-1α was present. Monocytes incubated with rhuIFN-γ plus MDP in the presence of rhuIL-4 showed a significant reduction in the secretion of both IL-1α and IL-1β at 24 h and IL-1α at 48 h (Table 2).

DISCUSSION

In this study, we evaluated the effect of rhuIL-4 on human monocyte function in vitro. We first tested the ability of rhuIL-4 to activate human monocyte tumoricidal activity. Monocytes from 8 normal donors consistently failed to show cytotoxic activity following incubation with a broad dose range of rhuIL-4 but could be stimulated to lyse tumor cells by other monocyte activators. Even after costimulation with triggering doses of LPS or MDP or priming doses of rhuIFN-γ, the rhuIL-4 consistently failed to stimulate tumoricidal activity (Table 1). We therefore concluded that rhuIL-4 did not activate the antitumor properties in normal monocytes.

To determine the influence of rhuIL-4 on human monocyte-mediated tumoricidal function in vitro, we incubated monocytes with rhuIL-4 plus a known monocyte activator, rhuIFN-γ plus MDP. Monocytes costimulated with rhuIFN-γ, MDP, and rhuIL-4 exhibited a dose-dependent reduction in monocyte-mediated cytotoxicity when compared to monocytes stimulated with rhuIFN-γ plus MDP alone (Fig. 1). This suppression correlated with a reduction in monocyte-mediated IL-1 production (Fig. 2). The availability of antisera to IL-1α and IL-1β enabled us to determine that rhuIL-4 inhibited the secretion of both IL-1α and IL-1β at 24 h and IL-1α at 48 h (Table 2).

Supernatants and cell lysates obtained from monocytes stimulated with LPS plus rhuIL-4 showed a similar reduction in IL-1 activity when compared to those obtained from monocytes incubated with LPS alone (Table 2). This reduction in LPS-stimulated IL-1 activity was due to inhibition of IL-1α and IL-1β secretion in the 24- and 48-h supernatants and cell lysate (Table 2).

Other investigators have also demonstrated the dose-dependent suppression of IL-1 synthesis by IL-4. Hurme et al. (24) showed that rhuIL-4 in doses as small as 50 units/ml totally inhibited LPS-stimulated monocyte IL-1 production by a posttranscriptional mechanism. Similarly, Te Velde et al. (27) demonstrated that rhuIL-4 reduced the secretion of cytokastic and chemotactic factors by monocytes and correlated this effect with a reduction in the “spontaneous” production of IL-1 activity by the monocytes.

IL-4 is known to participate with other cytokines in a complex regulatory network to influence the growth and differentiation of hematopoietic cells as well as to modulate the immune response. Murine IL-4 has been reported to stimulate macrophage tumoricidal activity. However, little is known about the effect of rhuIL-4 on human monocytes. Currently, rhuIL-4 produced by a strain of yeast containing a modified version of
the human IL-4 gene is undergoing clinical studies to determine its efficacy in the treatment of cancer. We report that rhuIL-4 did not directly stimulate normal donor monocyte antimelanoma properties in vitro. In fact, rhuIL-4 reduced monocyte-mediated IL-1 production and secretion. Augmenting immune response by increasing the antitumor activity of effector cells is the objective of many therapeutic trials. Further investigation of IL-4 and its role in the cytokine network is therefore essential to developing effective immunotherapy for cancer patients.

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