Susceptibility of Monocytes to Lymphokine-Activated Killer Cell Lysis: Effect of Granulocyte-Macrophage Colony-Stimulating Factor and Interleukin-3

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Cultured human monocytes have been shown to be susceptible to lysis by autologous lymphokine-activated killer (LAK) cells. To determine factors that might modulate the sensitivity of monocytes to lysis, we cultured adherent peripheral blood leukocytes (PBL) in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) or interleukin-3 (IL-3) since these cytokines have been reported to affect both functional and physical characteristics of monocytes. Both recombinant human GM-CSF and IL-3 were found to significantly enhance the susceptibility of monocytes to lysis by LAK cells in a dose-dependent manner, with GM-CSF being slightly more effective. In a kinetics study, the lysis ability of monocytes increased after two days of incubation with either cytokine, with maximal susceptibility occurring after four to six days of culture. The effects of GM-CSF and IL-3 appeared to be specific for monocytes since culture of either nonadherent cells or granulocytes, which are normally resistant to LAK-mediated lysis, did not induce sensitivity. While the effects of GM-CSF and IL-3 have been shown to be synergistic in some cases, they did not act synergistically to induce monocyte susceptibility to LAK lysis. In cold target experiments cytokine-treated monocytes reciprocally blocked lysis, suggesting that similar target structures were modulated with either factor. FACS analysis and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated comparable modulation of surface antigens with either GM-CSF or IL-3. Thus, these cytokines can serve to augment susceptibility of monocytes to LAK cells, emphasizing the complex interactions that occur in the immune system.

Studies in vivo and in vitro have established the existence of a group of glycoproteins, called colony-stimulating factors (CSF), that are characterized by their ability to induce the proliferation and differentiation of hematopoietic progenitor cells. It has also become evident that these CSF can enhance the effector functions of mature myeloid lineage cells. For example, human monocyte cytotoxicity can be activated by granulocyte macrophage (GM)-CSF and interleukin-3 (IL-3),2 apparently via induction of tumor necrosis factor (TNF) release. These cytokines are also active in enhancing antimicrobial activity in macrophages. GM-CSF and M-CSF have been shown in separate reports to enhance murine macrophage function against Leishmania tropica4 and Candida albicans. Of the CSF, IL-3 appears to have the ability to induce the widest range of hematopoietic cell proliferation and differentiation. Moreover, IL-3 can synergistically increase the number of G-CSF- or GM-CSF-induced colonies from human bone marrow. Less is known of functional activation by IL-3, but it is reported to stimulate the growth of murine mast cells/basophils and to enhance their cytolytic activity against WEHI-164 tumor cells.

Human clinical trials have been promising. Groopman et al9 have reported that GM-CSF infusions ameliorated some of the leukopenia commonly seen in patients with acquired immunodeficiency syndrome (AIDS) or the AIDS-related complex. Patients with myelodysplastic syndromes responded similarly in a study by Vadhan-Raj et al. Furthermore, cancer patients who had received high-dose chemotherapy and autologous bone marrow transplants, responded to GM-CSF by increased levels of leukocytes.11 Thus, the CSF family of hormones are potent candidates for the treatment of diseases that are associated with leukopenias.

One cancer treatment modality that has shown some promise is adoptive immunotherapy using lymphokine-activated killer (LAK) cells and IL-2 administration. Rosenberg et al,12 using LAK cells generated from the peripheral blood of 25 patients with advanced melanoma, renal, colon, and lung carcinoma, reported objective responses to LAK/IL-2 therapy. Their updated report on 157 patients with metastatic cancer indicates that, at least for some patients, LAK/IL-2 immunotherapy arrested tumor progression.13 However, severe toxicities associated with high-dose IL-2 and LAK therapy have been reported,14 including chills, fever, malaise, headaches, nausea, lymphopenia, and anemia.

In an attempt to understand the toxicity and range of targets recognized by human LAK cells, we recently identified autologous normal monocytes to be selectively lysed by these effector cells.16 The recognition was selective only for monocytes in the circulating WBC pool because polymorphonuclear leukocytes (PMN) and lymphocytes resisted lysis even after four to seven days in culture. On the other hand, in vitro culture of monocytes increased their susceptibility to LAK lysis, with optimal sensitivity detected four to five days after culture. The increased susceptibility may be related to in vitro differentiation of monocytes to macrophages. Becker et al17 carefully documented that human monocytes, cultured in vitro in human AB serum, develop a number of macrophage differentiation markers, which can be further enhanced by M-CSF or GM-CSF.

Recognition and lysis of monocytes by LAK cells may have biological consequences that might contribute to some of the side effects seen with LAK/IL-2 adoptive immunotherapy. To identify factors that might influence the sensitiv-
ity of monocytes to LAK lysis, and thus provide clues to the antigenic structure(s) on monocytes recognized by LAK cells, we cultured human monocytes in the presence of GM-CSF and IL-3. The present study demonstrates that these cytokines render monocytes significantly more susceptible to LAK lysis than monocytes cultured in medium alone.

**MATERIALS AND METHODS**

**Preparation of human leukocytes.** Leukocyte buffy coats, obtained from normal volunteers at the Southwest Florida Blood Bank, were diluted 1:2 in phosphate buffered saline (PBS) (M.A. Biologics, Walkersville, MD) and layered on 10 mL of Ficoll-Hypaque solution (Pharmacia, Piscataway, NJ). After centrifugation at 400 g for 20 minutes at room temperature, the band of peripheral blood leukocytes (PBL) at the interphase was collected and washed twice with PBS. PMN were collected by aspirating the band of cells lying on the surface of the RBC pellet. RBC were lysed by the addition of sterile distilled water for 20 seconds followed by 10 x PBS to a final concentration of 1 x PBS. PMN were then washed twice in PBS. The washed PBL and PMN were suspended in RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) containing 5% heat-inactivated human AB serum (Flow Laboratories, McLean, VA), 2 mmol/L L-glutamine, 10 U/mL penicillin, 100 μg/mL streptomycin, 5 mmol/L HEPES buffer (GIBCO), and 5 x 10^{-4} mol/L 2-mercaptoethanol (Sigma Chemical Co, St Louis), and will subsequently be referred to as complete medium. Plasticware was purchased from COSTAR (Cambridge, MA). Recombinant human GM-CSF (specific activity of 4 x 10^{10} colony-forming units [CFU] per milligram of protein) and recombinant human IL-3 (sp act, 4 x 10^{6} cfu/mg) were very generous gifts from Immunex Corp, Seattle.

**Preparation of monocytes.** PBL were allowed to adhere to tissue culture flasks for one hour at 37°C. Nonadherent cells (NAC) were recovered by vigorous washing of the flasks with warm medium, and the adherent cells were cultured for one to seven days with fresh medium, as previously described. Adherent cells were removed by gentle scraping with a cell scraper (COSTAR) after the addition of sterile distilled water for 20 seconds followed by 10 x PBS to a final concentration of 1 x PBS. PMN were collected by aspirating the band of cells lying on the surface of the RBC pellet. RBC were lysed by the addition of sterile distilled water for 20 seconds followed by 10 x PBS to a final concentration of 1 x PBS.

**Discontinuous Percoll density gradient centrifugation.** The separation of large granular lymphocytes (LGL) from T cells was accomplished by the use of a discontinuous Percoll density gradient. NAC were further depleted of adherent cells and B cells by incubation on nylon wool columns for 30 minutes at 37°C. The cells passing through the columns were then placed on a six-step discontinuous density gradient with a range from 40% to 52.5% Percoll, as previously described. After centrifugation at 550 g for 30 minutes at room temperature, the bands of lymphocytes were collected and examined for LGL morphology on Giemsa-stained cytocrifuged slides. In this series of experiments, fractions 2 and 3 contained 60% to 80% LGL and represented 10% to 15% of PBL.

**Activation of effector cells.** LGL from Percoll fractions 2 and 3 were incubated at a concentration of 2 x 10^{6} cells/mL in 5 to 10 mL volumes with 100 U/mL of recombinant human IL-2, which was kindly provided by Hoffman-LaRoche, Inc (Nutley, NJ), as previously described. Cells were optimally activated for three to four days in 25 cm² tissue culture flasks, and were then washed twice in cold medium and readded to the original cell concentration.

**FACS analysis of monocytes.** Monocytes were cultured in the presence or absence of 1,000 U/mL of GM-CSF or 1,000 U/mL of IL-3 for four days, recovered, washed in PBS, and labeled with 40 μL of the indicated monoclonal antibody per 10^{6} cells. Cells were then indirectly labeled with fluorescein-conjugated goat anti-mouse Ig and analyzed on the FACSscan flow cytometer (Beckton-Dickinson, Mountain View, CA). Reagents were obtained from Beckton-Dickinson, unless specified. Anti-HLA-A,B,C antibodies were obtained from AMAC, Inc (Westbrook, ME) and FcgRI (clone 32.2), FcgRII (clone IV-3), and FcgRIII (clone 3G8) were a generous gift from Dr M.W. Fanger (Dartmouth Medical School, Hanover, NH).

**SDS-PAGE analysis of monocyte membrane proteins.** For each cytokine, 5 x 10^{6} monocytes were treated as described, washed in PBS, and suspended in disruption medium containing 50 mmol/L Tris-HCl, pH 8.0, with 0.1 mmol/L PMSF and 1 mmol/L EDTA. Cells were sonicated using a Branson sonicator at 40% maximal energy with three 10-second bursts. Cells were confirmed to be lysed by examination using trypan blue exclusion. Membranes were collected and washed twice by centrifugation at 17,000 g at 4°C in the disruption medium. The membranes were then solubilized by incubation at 4°C for one hour in a buffer containing 10 mmol/L Tris-HCl, pH 7.8, 140 mmol/L NaCl, 10 mmol/L CHAPS, and 0.1 mmol/L PMSF and 1 mmol/L EDTA as protease inhibitors. Particulate debris was removed by centrifugation at 16,000 g for ten minutes and the supernatants were treated with SDS and 2-mercaptoethanol as described.

**Measurement of cytotoxicity.** A five-hour ¹¹⁶Cr-release assay was used to measure the cytotoxicity of IL-2-activated LGL against autologous cytokine-treated or untreated monocytes. Monocytes were labeled with 400 μCi of sodium [¹¹⁶Cr] chromate (Amersham Corp, Arlington Heights, IL) for two hours in 0.5 mL of medium, as described for fresh tumor targets. The cells were then washed twice and incubated an additional 30 minutes in 5 mL of medium. Target cells were then washed twice more and then added to effector cells at 5 x 10^{6} cells/well, resulting in effector:target ratios ranging from 20:1 to 2.5:1 in a final volume of 0.2 mL in each well. After five hours incubation at 37°C, the culture supernatants were harvested by removing 0.1 mL of the fluid to be counted in a gamma counter. Maximum isotope incorporated was determined by counting target cells alone, and spontaneous release was measured by counting supernatants of targets incubated with medium alone. The percentage of specific lysis was calculated by the formula: [(experimental cpm – spontaneous cpm)/maximal cpm incorporated] x 100. All determinations were done in triplicate. The SEM of all assays was calculated and was typically 5% of the mean or less. Lytic units (LU) were calculated and were defined as the number of effector cells required to lyse 20% of 5 x 10^{6} target cells. Paired t tests were performed to determine significant differences between the lysis of cytokine-treated and untreated monocyte target cells.

**RESULTS**

**FACS analysis of cytokine-treated monocytes.** To determine the effects of GM-CSF and IL-3 on the phenotype of treated monocytes, FACS analysis was performed on IL-3- and GM-CSF-treated and untreated monocytes using a panel of monoclonal antibodies that identify various monocyte and macrophage antigens. The results are shown in Fig 1 and 2. After incubation with GM-CSF or IL-3, monocytes had a higher expression of HLA-DR, which is an indication of the activation status of monocytes. On the other hand, expression of HLA-A,B,C, which is MHC class I antigen,
Fig. 1. FACS analysis of monocytes. I. MHC antigens. Monocytes were cultured in medium alone or in the presence of 1,000 U/mL of GM-CSF or IL-3 for four days before being stained with the indicated monoclonal antibodies for FACS analysis. Control graphs represent analysis of monocytes with the indirect stain, FITC-labeled goat antimouse antibodies, alone. Abscissa is the number of cells counted and the ordinate is the intensity of stain as measured by relative fluorescence units (RFU). (X indicates mean RFU).

was slightly decreased after incubation with either cytokine. As seen in Fig 2, the expression of Leu M5, which is a tissue macrophage marker, is markedly enhanced, indicating that both GM-CSF and IL-3 induced differentiation of monocytes to macrophage-like cells in culture. Also the expression of all three Fe-gamma receptors were likewise enhanced. These receptors bind different forms of monomeric and oligomeric IgG and appear to play a critical role in triggering effector functions of human monocytes. Thus, GM-CSF and IL-3 appeared to similarly modulate several of the differentiation markers and class I and II MHC antigens on monocyte/macrophages.

Dose response to GM-CSF and IL-3. In preliminary experiments using a variety of cytokines to determine their effects on the susceptibility of monocytes to lysis by autologous LAK cells, we found that monocytes, cultured for three days in the presence of 1,000 units of GM-CSF per milliliter, were more sensitive to LAK lysis than monocytes cultured in medium alone. To further examine this effect, various dilutions of GM-CSF were added to monocyte cultures, incubated for four days, and the cells were assessed for their susceptibility to LAK lysis in a five-hour 51Cr-release assay. Recombinant human IL-3 was also assessed for its effect since this cytokine has similar properties to GM-CSF. The results are shown in Fig 3. Monocytes cultured in medium with 1,000 U/mL of GM-CSF or IL-3 were significantly more sensitive to lysis than monocytes cultured in medium alone, with as little as 10 U/mL eliciting some response. The induction of maximal sensitivity to lysis occurred after treatment of monocytes with 1,000 to 3,000 U/mL of GM-CSF or IL-3, with GM-CSF being slightly, though not significantly, more efficient.

Finally, cytokine-treated monocytes were not lysed by fresh LGL or LGL that had been incubated in medium alone without IL-2 (data not shown). Thus, IL-2 was required to induce LAK activity against IL-3- or GM-CSF-treated monocytes and cytokine treatment did not render monocytes susceptible to spontaneous NK activity associated with fresh LGL.

Kinetics of cytokine-mediated effects on monocytes. To determine the period of incubation with GM-CSF or IL-3 required to induce enhanced susceptibility, experimental cultures of monocytes were incubated for six days and 1,000 U/mL of GM-CSF or IL-3 was added at various times during their culture. As shown in Fig 4, monocytes that had been cultured in the presence of either cytokine for four to six days acquired maximal sensitivity to LAK lysis. Monocytes cultured for six days in medium alone or to which GM-CSF or IL-3 was added for the last day of incubation did not demonstrate increased susceptibility. Thus, at least two days of incubation with either cytokine was required to induce sensitivity. Monocytes appeared to respond more rapidly to GM-CSF than IL-3 although levels of susceptibility after six days of stimulation with these factors were similar.

Specificity of the effect of GM-CSF and IL-3. Since both cytokines are known to modulate functional activity and differentiation of both monocytes and granulocytes, the effect of GM-CSF and IL-3 on the susceptibility of granulocytes to LAK lysis was of interest. We have previously reported that granulocytes were not susceptible to LAK-mediated lysis, in contrast to monocytes, but treatment with GM-CSF or IL-3 might alter this resistance. The results in Table I indicate that treatment of granulocytes with 1,000 U/mL of GM-CSF or IL-3 did not render these
Fig 2. FACS analysis of monocytes. II. Monocyte/macrophage antigens. Monocytes were cultured in medium alone or in the presence of 1,000 U/mL of GM-CSF or IL-3 for four days before being stained with the indicated monoclonal antibodies for FACS analysis.

cells susceptible to lysis, even after three days of incubation. It should be noted that the viability of cytokine-treated and untreated granulocyte suspensions was 85% or greater after incubation. To further define the specificity of the effects of GM-CSF and IL-3, NAC, which were recovered after plastic adherence of PBL to remove monocytes, were also incubated with either cytokine for three days. As shown, both treated and untreated NAC were resistant to LAK-mediated lysis.

Cold-target inhibition of LAK activity. In an effort to examine the mechanism of lysis of cytokine-treated monocytes, cold-target inhibition studies were performed. Unlabeled cells were added to cytotoxicity assays to determine whether competition occurred with radiolabeled target cells for recognition sites on LAK effector cells. As shown in Fig 5, unlabeled monocytes effectively blocked the lysis of radiolabeled, untreated monocytes, but only minimally blocked the lysis of cytokine-treated monocytes. Cold GM-CSF-treated monocytes were found to successfully compete with all targets tested, including IL-3-treated monocytes. Likewise, cold IL-3–treated monocytes comparably blocked the lysis of GM-CSF– or IL-3–treated monocytes and untreated monocytes.

Lack of synergy between GM-CSF and IL-3. Since IL-3 and GM-CSF synergize with respect to their ability to induce colony formation from bone marrow the possibility that these cytokines synergistically enhance the susceptibility of monocytes to LAK lysis was examined. Monocytes
Fig 3. Dose response of monocytes to GM-CSF and IL-3. Monocytes were incubated for four days in the presence of the indicated concentration of either GM-CSF (○) or IL-3 (●) before being used as targets for LAK cells. The mean ± SEM of triplicate determinations is shown. Asterisks indicate statistically significant (P < .05) determinations compared with control target cells cultured in the absence of GM-CSF or IL-3.

Fig 4. Time response to cytokine treatment. Monocytes were incubated for six days and 1,000 U/ml of GM-CSF (○) or IL-3 (●) were added at various times during culture. Days of incubation indicates length of time that monocytes were incubated in the presence of cytokines during the six-day culture. Asterisks indicate statistically significant (P < .05) determinations compared with control target cells cultured in the absence of GM-CSF or IL-3 (0 days of incubation).

Table 1. Specificity of Effect of GM-CSF and IL-3

<table>
<thead>
<tr>
<th>Target</th>
<th>Treatment</th>
<th>% Specific Lysis ± SEM at E:T Ratios of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20:1</td>
</tr>
<tr>
<td>Monocyte</td>
<td>None</td>
<td>12.7 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>GM-CSF</td>
<td>20.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>IL-3</td>
<td>19.4 ± 1.9</td>
</tr>
<tr>
<td>NAC</td>
<td>None</td>
<td>−0.9 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>GM-CSF</td>
<td>−0.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>IL-3</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>PMN</td>
<td>None</td>
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</tr>
<tr>
<td></td>
<td>GM-CSF</td>
<td>−1.7 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>IL-3</td>
<td>−1.1 ± 0.9</td>
</tr>
</tbody>
</table>

Cells were incubated with the indicated cytokine at 1,000 U/ml for three days before their use as target cells for LAK cells. Shown is a representative experiment of four that were performed with similar results.
Table 2. Effect of IL-3/GM-CSF Combinations on Monocyte Susceptibility

<table>
<thead>
<tr>
<th>GM-CSF (U/mL)</th>
<th>IL-3 (U/mL)</th>
<th>% Specific Lysis + SEM at E:T Ratios of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20:1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>16.3 ± 1.3</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>15.9 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>18.7 ± 1.1</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>16.9 ± 1.2</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>20.1 ± 0.3</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>14.5 ± 1.4</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>20.0 ± 1.1</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>18.3 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>21.5 ± 0.7</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>29.6 ± 2.5</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>27.1 ± 1.2</td>
</tr>
</tbody>
</table>

Monocytes were incubated with the indicated cytokine at the indicated concentration for four days before use as target cells for LAK effector cells. Shown is a representative experiment of three that were performed with similar results.
increased. In addition, novel bands appeared in the cytokine-treated monocytes, particularly within the high molecular weight range. Thus, the effect of IL-3 and GM-CSF on surface proteins of monocytes was twofold; some were expressed at a higher level than in untreated cells, and some novel molecules were also detected.

**DISCUSSION**

Our recent identification of normal human monocytes as LAK targets has prompted us to define conditions that can modulate the susceptibility of these cells to lysis. The reason for defining parameters for modulation of target cell sensitivity is twofold: (1) to find means to protect monocytes from LAK-mediated lysis, and (2) to shed some light on the nature of the monocyte surface structure(s) that form the ligand(s) for LAK recognition. Because monocytes are known to be differentiated by a number of cytokines including interferons (IFNs) and CSF, and such differentiation is usually accompanied by alteration in cell surface antigen expression, we tested the ability of these cytokines to alter monocyte susceptibility to LAK lysis. We observed that either enhanced or decreased regulation of monocyte sensitivity could be achieved, depending on the cytokine used. We first reported that culture of monocytes with IFN-gamma induced resistance to LAK lysis. Induction of resistance was detectable with as little as 10 U/mL of recombinant human IFN-gamma, and the kinetics of induction was rapid, with resistance appearing within two hours of IFN-gamma exposure. Once resistance was induced, the inability of treated monocytes to be lysed by LAK cells lasted at least three days even in the absence of IFN.

In the present study, we report that GM-CSF and IL-3 have the opposite effect on monocytes, with susceptibility significantly increased after two days of culture in the presence of these cytokines. Peak susceptibility was reached after four to five days of culture in 1,000 U/mL of either CSF. These conditions coincided with the kinetics and the presence of these cytokines. Peak susceptibility was reached after four to five days of culture in 1,000 U/mL of either CSF. These conditions coincided with the kinetics and the presence of these cytokines. In other functional studies, similar doses of GM-CSF and IL-3 that induced monocyte susceptibility to LAK lysis also activated human monocytes to kill *C. albicans* (Wang et al, manuscript submitted). These results indicated that increased sensitivity to lysis was not due to instability of treated cells since their fungicidal capability, as well as tumoridical activity, is enhanced by similar treatment with these cytokines.

Our FACS analysis using Leu M5 demonstrated that GM-CSF and IL-3 induced a higher expression of this macrophage antigen than medium alone. It is tempting to speculate that some antigen(s) selective for differentiated macrophages may serve as LAK recognition structure(s). This is perhaps not an unreasonable hypothesis since circulating PMN, which are also known to respond to CSF, could not be induced by IL-3 or GM-CSF to become lysable by LAK cells. Although PMN are considered to be end-stage cells and may not proliferate in the presence of CSF, a number of antigens can be induced, eg, FMLP receptors. Our study indicates that LAK cells are not capable of recognizing PMN even when stimulated with GM-CSF or IL-3 for up to three days. This is also true of lymphocytes that were not modified by these cytokines.

The putative macrophage antigen recognized by LAK cells is, at present, unknown. GM-CSF is reported to upregulate FcRII, which has a low affinity for monomeric IgG but avidly binds aggregated IgG2b, on the surface of U937 cells. More importantly, this cytokine has been shown to increase FcRI, Leu M3 (CD14), Leu M5, OKM1 (CD11c), and HLA-DR on human monocytes in a four-day culture in the presence of human AB serum. Our own analysis of surface markers induced by IL-3 or GM-CSF using flow cytometric techniques has confirmed that IL-3 can induce the same types of increases in the surface antigens of monocytes as can GM-CSF, ie, MHC class II, FcRs, and Leu M5. Because of the similarities between IL-3 and GM-CSF in parallel induction of monocyte/macrophage surface antigens and susceptibility to LAK lysis, it appears that IL-3 acts via the same mechanism as GM-CSF and may mature monocytes among the same pathway. This speculation is supported by the similarities in proteins appearing in cytokine-treated monocyte surface membranes as demonstrated by SDS-PAGE. Additionally, the lack of synergy between IL-3 and GM-CSF in the induction of monocyte susceptibility to LAK lysis tends to suggest a common pathway in response to these factors. Interestingly, the class I MHC expression of monocytes decreased during culture with GM-CSF or IL-3 in our hands. In other studies, decreased MHC expression was correlated with augmented NK susceptibility of target cells, although a direct causal link has not been firmly established. Regardless, the correlation between decreased class I MHC expression and enhanced susceptibility to lysis can be extended to human monocytes treated with these cytokines.

Further analysis of the monocyte/macrophage target structure(s) recognized by LAK cells was performed by cold-target inhibition assays. GM-CSF-treated monocytes blocked the lysis of both treated and untreated monocyte targets. In parallel, IL-3-treated monocytes were similarly effective in their ability to serve as cold targets. Normal, untreated monocytes could block LAK lysis, but to a much lesser degree against cytokine-treated monocytes. These results suggest that IL-3 and GM-CSF either (1) upregulate the expression of a target structure on normal monocytes, or (2) induce the expression of a novel antigen that is also recognized by LAK cells. Either explanation is possible since FACS analysis and SDS-PAGE have indicated that both effects occur after treatment of monocytes with GM-CSF or IL-3. Thus, the putative target structure may be a normal differentiation antigen that is enhanced by IL-3 and GM-CSF or a new antigen that is induced de novo. Further study is required to answer these questions and may lead to better understanding of target structures on tumor or allogeneic target cells.

The clinical relevance of enhanced susceptibility of CSF-treated monocytes to LAK lysis may be of considerable interest. While CSF have been reported to alleviate the leukopenias associated with AIDS, chemotherapy, and myelodysplasias, the relationship between CSF and other biological response modifiers is not known. One side-effect of
IL-2/LAK adoptive immunotherapy is severe anemia, often accompanied by leukopenia, indicating an involvement in hematopoiesis. Using bone marrow cultures, IL-2 was shown to directly inhibit GM-CSF–induced colony formation, and in other studies, IL-2–activated PBL also suppressed G-M colony formation. While these LAK cells had low direct cytolytic activity against normal bone marrow cells, incubation with CSF may render these cells very sensitive to lysis, much like the response seen with monocytes. Thus, further studies are required to clearly define cytokine interactions, particularly with regard to clinical trials using multiple biological response modifiers.

REFERENCES

Susceptibility of monocytes to lymphokine-activated killer cell lysis: effect of granulocyte-macrophage colony-stimulating factor and interleukin-3

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