Enhancement of Murine Lymphokine-activated Killer Cell Activity by Retinoic Acid

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ABSTRACT

Since retinoids have been suggested to be capable of potentiating immunity, the present study was undertaken to determine the effect, if any, on lymphokine-activated killer (LAK) cell activity by retinoid acid, an active metabolite of vitamin A and a differentiation enhancer. Retinoic acid alone was shown to induce no cytotoxicity generated from nylon wool-treated nonadherent mural (BALB/c) splenocytes against natural killer-resistant, LAK-sensitive syngeneic target tumors. When combined with human recombinant interleukin-2 (IL-2), retinoic acid augmented LAK cell activity in both a dose- and time-dependent manner. The augmentation was detected at 10^-10 M retinoic acid and reached the maximum at 10^-7 M, a >200% increase in lytic activity. Kinetic study revealed that retinoic acid augmented significantly LAK cell activity when incubated in IL-2-containing culture as short as for 6 h before cytotoxicity was measured. The removal of retinoic acid from culture resulted in the loss of the augmentation. Retinoic acid was found to augment LAK cell activity in a wide range of IL-2 concentrations (750-12,000 IU/ml), even at 6,000 IU/ml where the maximal induction of LAK cell activity had been reached. No phenotype or proliferation of LAK cells was altered by the addition of retinoic acid to IL-2-containing culture. However, cellular serine protease activity, measured as Nα-acetyl-L-lysine thiochrysyl-esterase, in LAK cells was increased by retinoic acid also in a dose- and time-dependent manner. The enhancement in LAK serine protease activity was significantly correlated with that of augmented LAK cell activity. Overall these results demonstrated that IL-2-induced LAK cell activity was enhanced by retinoic acid and that the augmentation may be mediated by means of enhanced expression of cellular serine protease activity. This study also suggests that, in addition to its use in chemoprevention of cancer, retinoic acid is of potential in adoptive immunotherapy.

INTRODUCTION

Retinoids, namely, natural and synthetic analogues of vitamin A, have been shown to exhibit a variety of diverse effects on the growth and differentiation of both normal and transformed cells (1). Evidence available has demonstrated that retinoids inhibit the development of preneoplastic transformation and decrease the formation of spontaneously developed, chemically induced, and virally induced animal tumors (2–5). The chemopreventive effect of retinoids on carcinogenesis in animal models has been suggested to result from its direct growth inhibitory and/or differentiation-promoting action on tumor cells and is attributed in part to the capacity of retinoids to potentiate the immunity in the hosts (6, 7). Mice treated with retinoids have been shown to possess an enhanced host-versus-graft reactivity and an increased resistance to tumor transplantation (8, 9). An enhancement in both in vitro and in vivo killer T-lymphocyte function and cell-mediated cytotoxicity has been shown as a result of retinoid treatment (9–11). Of the extensive investigation on LAK in recent years, no information regarding retinoids and IL-2-induced LAK cell activity is yet available. In the present study, we have investigated the effect of retinoic acid, an active metabolite of vitamin A and a differentiation-enhancing agent, on the generation of murine LAK cell activity.

We have established a spontaneously developed, syngeneic murine mammary adenocarcinoma cell line, designated JC, which is resistant to NK cell but susceptible to LAK cell lysis (12, 13). Because it is weakly immunogenic and highly tumorigenic, the major biological characteristics mimicking that of human disease, JC is a more suitable model for experimental immunotherapy than other chemically or virally induced mammary tumor models. Using JC as the target, we have demonstrated that retinoic acid was capable of exerting a positive effect in both a dose- and time-dependent manner on the generation of LAK cell activity from syngeneic BALB/c mouse splenocytes and that the enhanced LAK cell activity was correlated significantly with the increase in cellular serine protease activity of LAK cells. These results also suggest that, in addition to its possible use in chemoprevention of cancer, retinoic acid, and retinoids in general, may be of potential in adoptive immunotherapy.

MATERIALS AND METHODS

Recombinant IL-2. Human recombinant IL-2, produced from Escherichia coli, was kindly supplied by Cetus Corporation (Emeryville, CA) with a specific activity of 18 x 10^6 IU/mg. Endotoxin level was <0.01 ng/ml. The purity was >98% as examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Mice. Eight-12-week-old BALB/c female mice were used. The BALB/c mice were supplied by our institute's West Seneca Laboratory (West Seneca, NY). Histocompatibility testing by skin grafting was performed routinely to ascertain the maintenance of the line.

Target Cell. The JC tumor line was established from a spontaneously developed mammary adenocarcinoma in an aged virgin female BALB/c mouse (12). JC is an NK-resistant cell line but susceptible to LAK cell lysis (13). JC was maintained in RPMI 1640 with 10% fetal calf serum (GIBCO, Grand Island, NY).

Generation of LAK Cells. The generation of LAK cells had been described previously (14). Briefly, the spleens from BALB/c mice were harvested aseptically and minced in a stainless steel mesh to produce a single cell suspension. The erythrocytes were lysed osmotically with warm Tris-ammonium chloride buffer, 0.17 M, pH 7.2. The remaining splenocytes were passed through a column of nylon wool (Cellular Products, Buffalo, NY), and the nonadherent cells were incubated at 37°C in complete medium containing IL-2 under a moist atmosphere with 5% CO2 in culture flasks or 6-well plates (Falcon, Lincoln Park, NJ) at a cell concentration of 1 x 10^6/ml. The complete medium contained RPMI 1640 with 10% fetal calf serum, 0.1 mM nonessential amino acids, 0.1 mM sodium pyruvate, 5 x 10^-2 M 2-mercaptoethanol, 0.3 mg L-glutamine/ml, 100 μg streptomycin/ml, and 100 units penicillin/ml.

Generation of LAK Cells in the Presence of Retinoic Acid. The nylon wool-treated nonadherent splenocytes were prepared as described...
Augmentation of murine LAK activity by retinoic acid

RESULTS

Effect on IL-2-induced murine LAK cell activity by various concentrations of retinoic acid. The splenocyte preparations from BALB/c mice were treated with nylon wool and the nonadherent cells, which were devoid of macrophages and B-lymphocytes, were cultured in complete medium containing either IL-2 (3000 IU/ml) alone or IL-2 plus various concentrations of retinoic acid for 3 days. As shown in Fig. 1, the addition of retinoic acid to IL-2-containing culture resulted in an augmentation of LAK cell activity, and the augmentation was retinoic acid dose dependent. The more retinoic acid added to the IL-2-containing culture, the greater was the LAK cell activity; especially retinoic acid at 1 x 10^-7 and 1 x 10^-6 M and in IL-2 alone for 1 day (33 ± 8, 18 ± 6 versus 12 ± 5 lytic units), 2 days (117 ±15, 75 ± 11 versus 58 ± 7 lytic units), 3 days (457 ± 28, 308 ± 20 versus 199 ± 26 lytic units), 4 days (1112 ± 112, 819 ± 50 versus 599 ± 33 lytic units), and 5 days (1203 ± 91, 918 ± 55 versus 712 ± 27 lytic units) were all statistically significant (P < 0.05). Fig. 2 also revealed that augmentation of LAK activity by retinoic acid was in both a time- and dose-dependent manner.

Augmentation by retinoic acid of LAK cell activity induced with various concentrations of IL-2. Induction of LAK cell activity has been shown to be IL-2 dose dependent and to reach the maximum at high concentration of IL-2 (14, 15, 18). In this series of experiments, we determined whether or not retinoic acid could augment further the maximum LAK cell activity that had been induced by IL-2. Nylon wool-treated nonadherent mouse splenocytes were cultured in complete medium containing 1 x 10^-7 M of retinoic acid plus various concentrations of IL-2 for 3 days, and the LAK cell activity was measured. As shown in Fig. 3, the generation of IL-2-induced LAK cell activity was IL-2 dose dependent and reached its maximum activity at around IL-2 6,000 and 12,000 IU/ml. At 6,000 IU/...
Augmentation of Murine LAK Activity by Retinoic Acid

Determined daily for 5 consecutive days and expressed in lytic units. Columns, means of triplicate assays; bars, SEM.

Fig. 2. The time-dependent manner of augmentation on LAK cell activity by retinoic acid. Nylon wool-treated nonadherent BALB/c mouse splenocytes (1 × 10^6/ml) were prepared and cultured with IL-2 (3000 IU/ml) and IL-2 plus retinoic acid at 2 concentrations (1 × 10^7 and 1 × 10^8 M). LAK cell activity was determined daily for 5 consecutive days and expressed in lytic units. Columns, means of triplicate assays; bars, SEM.

Fig. 3. The enhancement of LAK cell activity induced with various concentrations of IL-2 by retinoic acid. LAK cell activity was generated from nylon wool-treated nonadherent BALB/c mouse splenocytes (1 × 10^6/ml) by incubation with various concentrations of IL-2 as indicated plus retinoic acid (1 × 10^-7 M) for 3 days. The LAK cell activity was determined and expressed in lytic units. Columns, means of triplicate assays; bars, SEM.

Also shown in Fig. 3 is that retinoic acid enhanced the LAK cell activities induced from a whole spectrum of IL-2 concentrations examined. The differences between LAK cell activities induced by IL-2 alone and that augmented by retinoic acid were all statistically significant (P < 0.05). The augmentation on LAK cell activities by retinoic acid averaged 200% and ranged from 185% for 12,000 IU/ml to 229% for 1,500 IU/ml.

Kinetic Analysis of Augmentation of LAK Cell Activity by Retinoic Acid. Fig. 4 illustrates the results obtained from the addition of retinoic acid (1 × 10^-7 M) to complete medium containing IL-2 (3000 IU/ml) at various time intervals (3, 6, 12, 24, 48, and 72 h) before the completion of a 3-day culture; i.e., 3, 6, . . . , 72 h before the 51Cr cytotoxicity assay, retinoic acid was added to the IL-2-containing medium and cocultured for 3, 6, . . . , 72 h. As shown, culture of retinoic acid with IL-2 as short as only for 6 h, i.e., by adding retinoic acid 6 h prior to the 51Cr release assay for LAK cell activity, resulted in a significant increase in LAK cell activity (257 ± 24 versus 188 ± 22 lytic units, P < 0.05). The greatest enhancement of LAK cell activity was detected when retinoic acid was added to the medium simultaneously along with IL-2 at the beginning of culture.

In another series of experiments, in which retinoic acid (1 × 10^-7 M) was added to the IL-2 (3000 IU/ml)-containing complete medium at the beginning of culture, then removed 2 days later, the LAK cell activity was measured. The remaining LAK cells in both retinoic acid-treated and the control (IL-2 alone) groups were washed. The cells from the control group (A) were added with fresh complete medium containing IL-2; while those from the retinoic acid-treated group were divided into 2 groups; one group (B) was continuously cultured in IL-2 alone and the other (C) was continuously cultured in IL-2 plus retinoic acid (Fig. 5). LAK cell activities were obtained daily from these 3 groups for the next 3 days. As shown in Fig. 5, the group (C) cultured in complete medium supplemented with IL-2 plus retinoic acid exhibited the highest induction rate of LAK cell activity among the 3 groups. Although quantitatively the initially retinoic acid-treated/removed group (B) always generated a greater LAK cell activity than that of the control (A), the induction rates (slopes) of LAK cell activity were parallel between A and B groups, indicating that the removal of retinoic acid from complete medium containing IL-2 resulted in the loss of augmentation in LAK cell activity.

Effect of Retinoic Acid on Proliferation of LAK Cells. Nylon wool-treated nonadherent splenocytes (1 × 10^6/ml) were cultured in complete medium alone, complete medium containing IL-2 alone, complete medium containing IL-2 plus retinoic acid for 3 days. Retinoic acid (1 × 10^-7 M) was added to the IL-2-containing culture at the time indicated prior to the end of 3 days of culture. The LAK cell activity was determined and expressed in lytic units. Control indicates the addition of DMSO (0.001%) alone, the solvent used in preparation of retinoic acid solution. Points, means of quadruplicate assays; bars, SEM.

Fig. 4. The effect of the length of incubation time with retinoic acid on LAK cell activity. LAK cell activity was generated from culturing nylon wool-treated nonadherent BALB/c mouse splenocytes (1 × 10^6/ml) with IL-2 (3000 IU/ml) for 3 days. Retinoic acid (1 × 10^-7 M) was added to the IL-2-containing culture at the time indicated prior to the end of 3 days of culture. The LAK cell activity was determined and expressed in lytic units. Control indicates the addition of DMSO (0.001%) alone, the solvent used in preparation of retinoic acid solution. Points, means of quadruplicate assays; bars, SEM.

Fig. 5. The effect of the removal of retinoic acid on LAK cell activity. Nylon wool-treated nonadherent BALB/c mouse splenocytes (1 × 10^6/ml) were cultured in complete medium containing IL-2 (3000 IU/ml) and IL-2 plus retinoic acid (1 × 10^-7 M) for 2 days. The LAK activity was measured. The remaining LAK cells from both IL-2 alone and IL-2 plus retinoic acid cultures were washed with complete medium 3 times. The retinoic acid-treated LAK cells were then divided into two groups. One group was cultured in IL-2 alone (B), and the other group was cultured in IL-2 plus retinoic acid (C). The LAK cell activity from the original IL-2 culture alone which continued to be cultured in IL-2 (A) and the newly divided 2 groups (B, IL-2; C, IL-2 plus retinoic acid) from initial retinoic acid-treated/removed group was measured daily for the next 3 days and expressed in lytic units. Points, means of triplicate assays; bars, SEM; except on day 5 when only the average of duplicate assays was available.

3015
retinoic acid (1 × 10⁻⁷ M), IL-2 (3000 IU/ml), and IL-2 plus retinoic acid. Then the cell density was measured daily for 5 consecutive days. As indicated in Table 1 retinoic acid did not alter the proliferation of LAK cells in comparison with the control and IL-2.

Effect of Retinoic Acid on Phenotype of LAK Cells. Cell surface markers were assessed on LAK cells generated from culture for 3 days in IL-2 (3000 IU/ml) alone and IL-2 plus retinoic acid (10⁻⁷ and 10⁻⁴ M). As summarized in Table 2, in comparison with the freshly prepared and uncultured nylon wool-treated nonadherent splenocytes, the culture with IL-2 alone and with IL-2 plus retinoic acid for 3 days resulted in a decrease in the percentage of cells expressing Lyt-2 and no change in cells expressing Thy-1.2 and L3T4 but an increase in the cells carrying asialo GM₁ and IL-2 receptor. However, no significant difference was detected between the phenotyping obtained from the cells cultured in IL-2 alone and those in IL-2 plus retinoic acid in expression of these cell surface markers.

Enhancement of LAK Cellular Serine Protease Activity by Retinoic Acid. Identical to the experiments described and presented in Figs. 1, 3, and 4, and instead of LAK cell activity in lytic units, serine protease activity measured as BLT-esterase activity was obtained from IL-2-induced and retinoic acid-augmented LAK cells. Fig. 6 illustrates that LAK cellular BLT-esterase activity was induced by retinoic acid in a dose-dependent manner. Similar to the augmentation of LAK cell activity, a detectable increase in LAK cellular BLT-esterase activity was found at 1 × 10⁻¹⁰ M of IL-2 (10.95 ± 0.36 BLT-esterase activity units, P < 0.05) in comparison with the control (9.57 ± 0.64 BLT-esterase activity units) and reached the maximum (15.72 ± 1.44 BLT-esterase activity units, P < 0.01) at 1 × 10⁻⁷ M DMSO (0.01%) at the highest concentration used in this set of experiments showed no effect on the enzyme activity.

Retinoic acid at 1 × 10⁻⁷ M was shown to enhance cellular BLT-esterase activity in LAK cells cultured from a wide range of IL-2, 750–12,000 IU/ml (Fig. 7). As shown, highly elevated LAK cellular BLT-esterase activity was induced by IL-2 at 6,000 or 12,000 IU/ml and retinoic acid was still able to enhance the highly elevated enzyme activity even to a significantly greater level (6,000 IU/ml, P < 0.05; 12,000 IU/ml, P < 0.01).

Kinetic study of the effect on LAK cellular BLT-esterase activity by retinoic acid is shown in Fig. 8. An apparent increase in enzyme activity (9.67 versus 9.22 BLT-esterase activity units) was detected after IL-2-induced LAK cells were incubated with retinoic acid (1 × 10⁻⁷ M) for only 6 h prior to the assay of enzyme activity. The enhancement in BLT-esterase activity was also in a time-dependent manner. The longer the LAK cells were cultured with retinoic acid, the greater was the cellular BLT-esterase activity expressed.

DISCUSSION

In the present report, we have investigated the effect of retinoic acid on murine LAK cell activity, using a human-like murine syngeneic, spontaneously developed, weakly immunogenic, highly tumorigenic, NK-resistant, and LAK-sensitive mammary adenocarcinoma as the target. Retinoic acid alone was shown to be incapable of inducing any cytotoxicity from nylon wool-treated nonadherent BALB/c mouse splenocytes. However, when used in combination with IL-2, retinoic acid was shown to augment IL-2-induced LAK cell activity in both a dose- and time-dependent manner.

Kinetic study demonstrated that a greatly enhanced cytotoxicity was detected when retinoic acid was added to the IL-2-containing medium at the early induction phase of LAK cell activity. The addition of retinoic acid to complete medium containing IL-2 at the beginning of culture resulted in the maximal augmentation of LAK cell activity. Although an enhancement was detectable from an incubation for only 6 h prior to cytotoxicity assay, a continuous presence of retinoic acid in culture is required for the maximal augmentation of IL-2-induced LAK cell activity. When retinoic acid was removed from the IL-2-containing culture, the induction rate of LAK cell activity was decreased to a level identical to that of IL-2 alone, i.e., a loss of the augmentation.

It has been reported that, when fed with vitamin A acetate-enriched diet, the splenocytes of Mycobacterium bovis-infected mice increased in vitro IL-2 production (19). To assess whether or not the augmentation of LAK cell activity by retinoic acid is mediated by an increase in IL-2 production in culture, a fixed and constant concentration of retinoic acid was added to the culture containing various concentrations of IL-2. The results indicated that retinoic acid (10⁻⁷ M) augmented LAK cell activity by an average of 2-fold over a wide range of IL-2 concentrations even at high IL-2 concentrations in which the maximal induction of LAK cell activity by IL-2 had already been reached. These data along with the fact that retinoic acid alone induced no cytotoxicity suggest that the enhanced effect of retinoic acid on LAK cell activity is not mediated by the induction of an increased in vitro IL-2 production.

Retinoic acid has been shown to increase IL-2 receptor on activated human thymocytes but not on peripheral blood lymphocytes (20). It has been suggested that retinoic acid may modulate an IL-2-dependent immune response, in part, by up-regulating the expression of IL-2 receptor (20). In our study, we have examined the IL-2 receptor as expressed by LAK cells cultured in medium containing IL-2 alone and IL-2 plus retinoic acid. Our results revealed that retinoic acid exhibited little, if any, effect on the expression of IL-2 receptor. Therefore, the enhanced effect of LAK cell activity by retinoic acid appears not to be mediated by an increase in the expression of IL-2 receptor.

The effect of retinoic acid on proliferation of the lymphocytes also was examined in our study because some investigators have reported that retinoic acid enhanced lymphocyte proliferation in response to mitogens (21, 22), although others have indicated no such enhancement of proliferative response (10, 23). Our results indicated that retinoic acid did not alter the proliferation

### Table 1 The effect of retinoic acid on proliferation of LAK cells

<table>
<thead>
<tr>
<th>Cultured in</th>
<th>Days in culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Complete medium</td>
<td>0.26 ± 0.03*</td>
</tr>
<tr>
<td>Retinoic acid, 10⁻⁷ M</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>IL-2, 3000 IU/ml</td>
<td>0.70 ± 0.05</td>
</tr>
<tr>
<td>IL-2 + retinoic acid</td>
<td>0.67 ± 0.06</td>
</tr>
</tbody>
</table>

* Nylon wool-treated nonadherent BALB/c mouse splenocytes (1 × 10⁶/ml) were cultured in complete medium alone, complete medium containing retinoic acid (1 × 10⁻⁷ M), IL-2 (3000 IU/ml), and IL-2 plus retinoic acid. The cell density (×10⁶/ml) was counted daily for 5 consecutive days by using a cytometer.

* The values represent means ± SEM from triplicate assays.
Table 2 The effect of retinoic acid on phenotyping of LAK cells

<table>
<thead>
<tr>
<th>Cultured in</th>
<th>Thy-1.2</th>
<th>Lyt-2</th>
<th>Asialo GM1</th>
<th>IL-2 receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete medium</td>
<td>77 ± 2</td>
<td>45 ± 1</td>
<td>25 ± 3</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>IL-2, 3000 IU/ml</td>
<td>82 ± 2</td>
<td>29 ± 4</td>
<td>27 ± 5</td>
<td>51 ± 4</td>
</tr>
<tr>
<td>IL-2 + retinoic acid, 10^-7 M</td>
<td>84 ± 2</td>
<td>23 ± 3</td>
<td>23 ± 4</td>
<td>58 ± 6</td>
</tr>
</tbody>
</table>

* Freshly prepared nylon wool-treated nonadherent splenocytes cultured in complete medium alone.

The values represent means ± SEM from triplicate assays.

Fig. 6. The effect of retinoic acid on LAK cellular serine protease activity. LAK cells were generated from nylon wool-treated nonadherent BALB/c mouse splenocytes (1 × 10^6/ml) with IL-2 (3000 IU/ml) for 3 days. Retinoic acid at various concentrations as indicated was added to IL-2-containing complete medium at the beginning of culture. LAK cellular BLT-esterase activity was measured at the end of the 3-day culture. Points, means of triplicate assays; bars, SEM.

Fig. 7. The effect of retinoic acid on LAK cellular serine protease activity cultured with various concentrations of IL-2. LAK cells were generated from nylon wool-treated nonadherent BALB/c mouse splenocytes (1 × 10^6/ml) with various concentrations of IL-2 as indicated plus retinoic acid (1 × 10^-7 M) for 3 days. LAK cellular BLT-esterase activity was determined. Columns, means of triplicate assays; bars, SEM.

Fig. 8. The effect of the length of incubation time with retinoic acid on LAK cellular serine protease activity. LAK cells were generated from nylon wool-treated nonadherent BALB/c mouse splenocytes (1 × 10^6/ml) with IL-2 (3000 IU/ml). Retinoic acid (1 × 10^-7 M) was added to the IL-2-containing complete medium at the time indicated prior to the end of the 3-day culture. LAK cellular BLT-esterase activity was determined. Control indicates the addition of DMSO (0.001%) alone, the solvent used in preparation of retinoic acid solution. Points, averages of duplicate assays.

Results were obtained from the culture with IL-2 plus retinoic acid, indicating that the combination of IL-2 with retinoic acid did not increase the IL-2-induced LAK precursor cells, T-cells and NK-like cells.

Yet, one biochemical parameter, namely BLT-esterase, a serine protease, activity in IL-2-induced LAK cells was found to increase profoundly by retinoic acid. Significantly, the increase in LAK cellular BLT-esterase activity by retinoic acid was correlated with that of the augmented LAK cell activity in lytic units. This conclusion was based upon the comparison of the results obtained from three different sets of experiments. First, both LAK cell activity (Fig. 1) and LAK cellular BLT-esterase activity (Fig. 6) were augmented by retinoic acid in a dose-dependent manner. An increase in both parameters was detected at 10^-10 M of retinoic acid and reached the maximum at 10^-7 M, with a highly significant coefficient of correlation of 0.997. Second, when retinoic acid was combined with various concentrations of IL-2 in culture, IL-2 plus retinoic acid groups always exhibited a greater LAK cell activity (Fig. 3) and a greater LAK cellular BLT-esterase activity (Fig. 7) than those of the IL-2 alone group. A highly correlated association (r = 0.961) was found between these two experiments. Last, but not least, kinetic analysis revealed that both LAK cell activity (Fig. 4) and LAK cellular BLT-esterase activity (Fig. 8) were augmented by retinoic acid in a time-dependent manner. An augmentation was detectable from an incubation of only 6 h prior to ^111Cr release and enzyme activity assays, and the maximal enhancement was found when retinoic acid was added at the beginning of culture. Again, a significant correlation (r = 0.964) between LAK cell activity and BLT-esterase activity was found between the results obtained from these experiments.

Data available in recent years have provided some circumstantial but compelling evidence in favor of the granule exocytosis pathway as an important mechanism underlying the killer cell-mediated cytotoxicity (24, 25). One potent lytic protein called perforin/cytolysin has been identified as a granule component and shown to damage the target cell membrane by forming transmembrane lesions (26, 27). Other major constituents of cytolytic granules involving in the lytic mechanism of cell-mediated cytotoxicity include a family of highly related serine proteases (28, 29). The expression of serine protease enzymes and its genes has been shown to be induced by IL-2 in profile of nylon wool-treated nonadherent murine splenocytes in culture containing IL-2 plus retinoic acid in comparison with IL-2 alone.

When the freshly prepared nylon wool-treated nonadherent mouse splenocytes were compared, the phenotypic examination using cell surface markers revealed a decrease in percentage of the cells carrying Lyt-2, no change in the cells carrying Thy-1.2 and L3T4, and an increase in the cells expressing IL-2 receptors and asialo GM1 in IL-2-containing culture. Similar
murine NK and nonspecific T-killer cells (30). Additionally, three retinoic acid receptors (α, β, and γ) have been identified in both mouse and human cells and tissues (31). These retinoic acid receptors are responsible for biological function of retinoic acid by mediating a mechanism of action similar to that of steroid/thyroid hormones and their receptors (32, 33). In light of these reports along with our data on enhancement of LAK cellular BLT-esterase activity by retinoic acid, it is likely that an increase in IL-2-induced expression of serine protease activity is the major mechanism underlying the augmentation of IL-2-induced LAK cell activity by retinoic acid.

In summary, our study has revealed that retinoic acid, an active metabolite of vitamin A and a differentiation-enhancing agent, augmented IL-2-induced LAK cell activity in vitro in both a dose- and time-dependent manner and was accompanied by an increase in serine protease activity in LAK cells. Our results therefore indicate that the augmentation may be mediated by an increase in IL-2-induced expression of serine protease activity. This study also suggests that, in addition to its use in chemoprevention of cancer, retinoic acid and retinoids may be of potential in IL-2-related immunotherapy.

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