Infiltration of Interleukin-2-inducible Killer Cells in Ascitic Fluid and Pleural Effusions of Advanced Cancer Patients

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ABSTRACT

Using ascitic fluid or pleural effusion obtained from 13 ovarian or metastatic breast cancer patients, we separated tumor cells from effusion-associated lymphocytes (EAL) with Percoll density centrifugation. Lymphocytes were incubated with recombinant interleukin 2 (IL-2) for 3–4 days and then assessed for tumoricidal activity in a 51chromium-release assay. The IL-2-activated EAL were found to lyse autologous fresh tumor cells, as well as allogeneic fresh tumor cells and FMEX tumor cells, a melanoma cell line which is resistant to natural killer cell activity but is sensitive to lysis by lymphokine-activated killer cells. There was little or no tumoricidal activity seen in freshly isolated EAL or in EAL which were cultured in medium without IL-2. Phenotypically, the IL-2-activated EAL were largely CD3−, although some cytolytic activity was found in CD3+ populations. Also, most activity was found in cells positive for CD2 (OKT11) and CD16 (Leu 11b), and negative for the monocyte marker Leu M3. These results indicate that activated cell types found in EAL were predominantly natural killer/lymphokine-activated killer-like with a small contribution from T-cells. Finally, EAL were readily activated by IL-2 in medium containing autologous effusion fluid, indicating that in situ activation of tumoricidal activity by IL-2 can occur in the face of potentially inhibitory substances or cells that may exist in the effusions. Direct introduction of IL-2 may therefore be a potential therapeutic modality of effusion-forming cancers.

INTRODUCTION

Recently, the use of IL-22 in the treatment of cancer has been under intense investigation. The ability of IL-2 to stimulate antitumor activity of NK cells and cytotoxic T-lymphocytes in vitro (1–6), coupled with successful IL-2 and adoptive cell therapy in several animal tumor models (7–11), has led to optimism for immunological manipulation in cancer patients for treatment of established tumors. Indeed, 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 recently updated report on 157 patients with metastatic cancer reaffirms that, for some of the patients for whom standard therapy has proved ineffective, immunotherapy with LAK cells plus IL-2 has arrested tumor progression (13). Other investigators, including West et al. (14), have also found some efficacy with combined LAK/IL-2 therapy in cancer patients. However, severe toxicities associated with high dose LAK/IL-2 therapy (12–14) have necessitated the search for alternative cell types that are more potent and can be administered in smaller numbers to produce comparable or better efficacy with possibly fewer side-effects.

Many human tumors contain an infiltration of lymphocytes.

Because a more favorable prognosis is associated with a greater magnitude of lymphocytes infiltrating some histological tumor types (15–18), a number of investigators have focused on the isolation of these TIL to examine the immunological recognition of and potency against the associated tumor cells. Results to date suggest that TIL from human tumors are primarily T-cells with a minor population of other lymphocytes and macrophages (19–22). Most TIL have low cytotoxicity against autologous tumor cells and undetectable NK activity but, like LAK cells, can be readily activated by IL-2 to proliferate and lyse tumor cells (23–28). Some show specificity against autologous tumor cells while others appear to be non-MHC restricted.

The report of better efficiency of IL-2-expanded TIL than LAK for in vitro lysis of autologous tumor cells (29), as well as in producing remission in mice bearing MC38 colon carcinoma simultaneously treated with cyclophosphamide and IL-2 (30), has provided further impetus to initiate similar trials in humans. Progress is, however, hampered by the technical difficulties associated with isolation and expansion of TIL. In some cases, prolonged expansion has been reported to select for CD4+ cells that are no longer tumoricidal (27).

Because of the toxicities associated with LAK/IL-2 therapy and the formidable task facing those attempting to generate TIL from solid tumor biopsies, our goal is to define selective tumor types already in the environment of lymphocytes that can be activated by IL-2 in situ. Pleural effusions and ovarian ascites present environments where both effector lymphocytes and tumor target cells coexist in a defined space. In ovarian carcinoma, several reports have demonstrated the correlation of high lymphocyte count within the tumor with improved patient survival (31, 32). Lymphocytes infiltrating the pleural or peritoneal cavities of patients with advanced cancer may be amenable to local stimulation with IL-2, obviating the need for adoptive cell therapy and i.v. high dose IL-2 administration, which tend to increase toxicities. This study therefore attempts to examine and characterize EAL from patients with ovarian carcinoma or metastatic pleural involvement, particularly in response to IL-2, against autologous tumor cells and other LAK tumor targets.

MATERIALS AND METHODS

Patient Population. Peritoneal effusions (ascites) or pleural effusions were collected from 13 newly diagnosed patients with advanced or metastatic cancer seen at the H. Lee Moffitt Cancer Center and Research Institute, Women's Diagnostic Center, University of South Florida College of Medicine, or at the Community Cancer Center, St. Joseph's Hospital, Tampa, FL. Five of the patients had metastatic breast carcinoma with pleural effusions, and the remaining eight women had ovarian carcinoma with ascites. The patients ranged in age from 25 to 67 years and were not under treatment with anticancer agents at the time of sample collection.

Preparation of Effusion Cells. Specimens of pleural effusions and ascites (500–4000 ml) were obtained from the patients and 20 ml of fluid was collected before 100 U per ml of sterile heparin (Sigma Chemical Co., St. Louis, MO) was added to prevent clotting. Effusions were then centrifuged at 400 × g for 10 min at 25°C to sediment cells.

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2 The abbreviations used are: EAL, effusion-associated lymphocytes; NK, natural killer; LAK, lymphokine-activated killer; IL-2, interleukin-2; TIL, tumor-infiltrating lymphocytes; MHC, major histocompatibility complex; PBS, phosphate buffered saline.

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Cells were washed with sterile PBS (M. A. Biologics, Walkersville, MD) and then layered on Ficoll-Hypaque cushions (Pharmacia, Piscataway, NJ). After centrifugation at 400 x g for 20 min, tumor cells and mononuclear cells were collected from the interface and washed twice in PBS. The washed effusion cells were then suspended in RPMI 1640 medium containing 5% heat-inactivated human AB serum (Flow Labs, McLean, VA), 2 mM glutamine, penicillin, and streptomycin, 5 mM 4- (2-hydroxyethyl)-1-piperazineethanesulfonic acid, and 5 x 10^{-3} M 2-mercaptoethanol, and will subsequently be referred to as complete medium. Where indicated, complete medium was supplemented with 5% hemar-free effusion fluid instead of human AB serum. Media reagents were purchased from GIBCO (Grand Island, NY) and plastic supplies were from CoSTAR (Cambridge, MA). The percentage of tumor cells ranged from 20 to 90% as judged by morphological examination of Wright-Giemsa stained cytocentrifuged preparations.

Separation of Tumor Cells from EAL. Tumor cells were separated from mononuclear cells by centrifugation on discontinuous Percoll density gradients according to the method of Uchida et al. (33), with modifications. Complete medium and Percoll (Pharmacia) were adjusted to 285 mOsmol/kg H2O with distilled water and 10X concentrated PBS, respectively. Four different concentrations of Percoll in medium were prepared (35.0, 41.7, 45.0, and 66.7%), and 3.5 ml of each concentration was carefully layered into 15-ml plastic conical tubes. On top of each gradient was placed 1 x 10^{6} effusion cells, and the gradients were centrifuged at 550 x g for 30 min. Cells banding at each layer were collected, washed, and suspended in complete medium. Typically, cells from Fraction 0 (at the interface of medium and 35% Percoll) were 80-95% tumor cells and were subsequently used as fresh tumor target cells. Fraction 1 (at the interface of 35% and 41.7% Percoll) was a mixture of tumor cells, mesothelial cells, and monocytes which was usually 5-15% of the total cell numbers. Fraction 2 (at the interface of 41.7% and 45% Percoll) contained typically 40-60% large granular lymphocytes with the remainder consisting of smaller lymphocytes, and contaminating monocytes or tumor cells at less than 20%. The majority of the small lymphocytes were concentrated at the interface of 45% and 66.7% Percoll (Fraction 3), and red blood cells were found in the pellet.

To further remove monocytes from lymphocytes, Fractions 2 and 3 were pooled and 5 x 10^{6} cells were incubated for 60 min at 37°C on plastic Petri dishes. Nonadherent cells were removed by vigorously washing the plates three times with warm complete medium. The viability of all fractions was greater than 95% according to the ability to exclude trypan blue. Fresh tumor cells (Fraction 0) were cryopreserved at −70°C in fetal calf serum containing 10% dimethyl sulfoxide until ready for use as target cells in the cytotoxicity assays, at which time cells were carefully thawed, slowly diluted in medium, and washed. Tumors were accepted for assay if viability was greater than 85%.

Activation of Effector Cells. Cells were incubated at a concentration of 2 x 10^{6} cells/ml in complete medium with 100 U of recombinant human IL-2/2/ml, kindly provided by Hoffmann LaRoche, Inc. (Nutley, NJ). Cells were activated for the indicated time in 25-cm^{2} tissue culture flasks, and were then washed twice in medium and readjusted to the original cell concentration.

Serological Depletion of Effector Cells. EAL, after activation with IL-2, were treated with various monoclonal antibodies with complemen- to identify the phenotype of the effector cell which lysed autol- ogous tumor cells. Cells were washed twice in plain RPMI and pelleted in culture tubes at 2 x 10^{6} per treatment (3). A previously determined optimal dilution of monoclonal antibody was added directly to cells in a final volume of 0.1 ml, and incubated for 20 min at room temperature. OKT reagents were purchased from Ortho Pharmaceutical Corp. (Raritan, NJ), and the Leu reagents were from Beckton-Dickinson Co. (Mountain View, CA). Antibodies to CD4 (OKT4) were used to lyse T-lymphocytes, CD8 (Leu 2a), anti-CD3 (OKT3) lyses all T-cells, and anti-CD2 (OKT11) is effective against most T-cells and NK cells. Anti-CD16 (Leu 11b) lyses fresh NK cells, and anti-Leu M3 lyzes monocytes/macrophages. Following antibody treatment, rabbit complement (Cedarlane, Westbury, NY) was added to cells at 1:10 dilution in a final volume of 1.0 ml and incubated for a 45 min at 37°C. Cells were examined for viability using trypan blue exclusion, washed twice in RPMI 1640, and then resuspended in complete medium to the original volume to avoid enrichment of interfering populations. The effector:target (E:T) ratio that is indicated was calculated from the number of viable cells that were present prior to treatment with antibody plus complement.

Immunofluorescence Column Separation of Effector Cells. Immunofluorescence columns were used in lieu of serological depletion to further examine the identity of the effector cell population. Protein A-Sepharose columns were prepared as described (34). IL-2-activated EAL were washed twice in PBS, incubated with 0.1 ml of antibodies per 10^{6} cells for 30 min at room temperature, and washed twice again in PBS. Antibody-labeled cells were then loaded onto Protein A-Sepharose columns with bed volumes of 2 ml. Nonadherent cells (antibody negative) were collected, washed twice, and resuspended in complete medium at 2 x 10^{6} cells/ml. The adherent cells (antibody positive) were recovered by agitation of the gel bed in combination with increased flow rates. About 70% of the cells which were loaded onto the columns were recovered in this manner.

Measurement of Cytotoxicity. A 4-h 51Cr-release assay was used to measure the cytotoxicity of IL-2-activated EAL against autologous or allogeneic fresh tumor targets and FMEX, which is an NK-insensitive melanoma cell line (35). FMEX was included as a target to control for activation conditions and as an indicator of LAK activity. Fresh tumor cells were labeled with 400 μCi of sodium [51Cr]chromate (Amersham Corp., Arlington Heights, IL) for 2 h in 0.5 ml of medium, as described (36). The cells were then washed twice and incubated an additional 30 min in 5 ml of medium. FMEX target cells were labeled with 200 μCi of [51Cr] per 10^{6} cells for only 1 h prior to assay. 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 5:1 in a final volume of 0.2 ml in each well. After 4-h 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 incorporation was determined by counting target cells alone, and spontaneous release was measured by counting supernatants of targets incubated with medium alone. The fresh tumor targets exhibited 10-25% spontaneous release during the 4-h assay and the spontaneous release from FMEX tumor targets was typically 5-10%. 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 5% of the mean or less. Lytic units 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 IL-2-activated and unactivated effector cells.

RESULTS

Lysis of Autologous Fresh Tumor Targets and FMEX. To demonstrate the ability of IL-2 to activate lysis of autologous fresh tumor target cells in a 4-h 51Cr-release assay, EAL were purified from cancerous effusions from 13 patients. Four of the patients (Patients 1, 5, 7, and 13) were diagnosed with metastatic breast carcinoma, and the remaining patients had ovarian carcinoma. Following Percoll density gradient centrifugation, purified EAL were activated with 100 U/ml of recombinant IL-2 for 4 days and then assessed for their cytotoxic activity against both FMEX, an NK-insensitive melanoma cell line, and against freshly thawed autologous tumor target cells. Fig. 1 represents the lysis of FMEX tumor cells by each of the IL-2-activated EAL, and Fig. 2 shows the lysis of their respec-
ineffective against autologous tumor cells, indicating that some tumor cells can be resistant to lysis regardless of the activation status of killer cells. In this regard, tumor cells from Patient 6 also resisted lysis by LAK cells generated from the peripheral blood of four normal donors, each of which efficiently lysed FMEX tumor cells (data not shown). It should also be noted that freshly collected pleural effusions and ascitic fluids both contained tumor cells that were conjugated to lymphocytes (from 16 to 55% of the tumor cells were bound to small lymphocytes prior to Percoll fractionation) but these tumor-binding lymphocytes were not cytotoxic when they were tested immediately against fresh tumor cells or FMEX (see below). EAL which were cultured for 4 days in medium without IL-2 also could not kill autologous tumor cells nor FMEX target cells. Thus, IL-2 was required for the induction of cytotoxic activity in EAL.

Kinetics of Activation of EAL. To examine the kinetics of activation of cytotoxic activity, EAL were incubated from 0 to 5 days in the presence or absence of IL-2. On each day, EAL were assessed for their antitumor activity against both freshly thawed autologous tumor cells and the FMEX tumor cell line. As shown in Fig. 3, little or no cytotoxicity was seen against either tumor target for 0, 1, or 2 days in culture, either in the absence or presence of IL-2. At Day 3 of IL-2 treatment, significant cytolysis activity was detected in EAL against both FMEX and autologous tumor cells. Lytic activity against fresh tumor cells peaked after 4 days of incubation with IL-2, and began to fall by Day 5 of culture. While antitumor activity against FMEX cells was consistently higher, IL-2-activation of tumoricidal activity in EAL paralleled that against fresh tumor cells. In the absence of IL-2 in the culture medium, no cytotoxicity was present against either target used. Fig. 3 is representative data of three patients that were assessed with similar results. While tumoricidal activity varied among patients (see Figs. 1 and 2), maximal lysis of fresh tumor cells occurred between Days 4 and 5 of culture of EAL with IL-2 under the conditions of our assay.

Lysis of Heterologous Fresh Tumor Cells. The ability of EAL to be activated by IL-2 to kill FMEX melanoma cells in addition
to autologous tumor cells suggested that the effector cells were non-MHC-restricted in their capacity to kill target cells. Thus, selected EAL were activated for cytotoxicity against heterologous fresh tumor cells, in addition to their autologous targets. The results are shown in Table 1. Typically, the EAL from each patient were seen to lyse heterologous fresh tumor cells. While the EAL from four of the five patients were more effective against autologous tumors, the susceptibility of tumor cells to lysis appeared to be constant, regardless of the effector cell used. These data suggest that killing of fresh tumor cells depended on at least two variables; the activation status of the effector EAL and the susceptibility of tumor cells to lysis.

**Percoll Fractionation of IL-2-activated EAL.** In an effort to identify and characterize the effector cell in EAL which kills tumor cells, Percoll density fractionation was performed on effusion cells. Cells from each fraction, as well as the whole effusion mixture, were cultured in the presence or absence of IL-2 for 4 days. Fractionated cells were then assessed for cytotoxicity against both FMEX and autologous fresh tumor cells. Table 2 summarizes the results from Patients 7, 9, 12, and 13. EAL from Patient 12 were activated by IL-2, with the majority of activated cells being found in Fraction 2 (F-2). Cells from Fraction 1, and Fraction 3 to a lesser extent, were also capable of lysing FMEX or autologous tumor cells. Interestingly, IL-2 was able to induce cytotoxic activity in the whole effusion mixture, suggesting that in situ activation of antitumor activity could occur. Percoll fractionation of EAL from Patient 9 resulted in a slightly different pattern of cytotoxic activity. While IL-2 did not induce much activity in either the whole effusion preparation or in Fractions 1 and 2, this cytokine was capable of activating Fraction 3 cells to kill both FMEX and autologous fresh tumor cells. It should be emphasized that unseparated whole effusions from Patients 9 and 13 contained high levels of tumor cells, i.e., 54 and 58% tumor cells, respectively. Removal of the tumor cells by Percoll fractionation prior to IL-2-activation enhanced LAK activity in Fraction 3 of Patient 9 and Fractions 2/3 of Patient 13 to a considerable degree. Little or no tumoricidal activity was seen in any of the fractions in the absence of IL-2 (data not shown).

**Effect of Adherent Cells on Cytotoxicity of IL-2-activated EAL.** The role of monocytes/macrophages in the regulation of lymphocyte cytotoxicity is less clear. While some reports have demonstrated the dependence of IL-2 induced lymphocyte activation on the presence of monocytes (37), other studies demonstrate the suppressive effects of adherent cells on NK and LAK activities (38, 39). Tumor-associated macrophages are even more controversial. Tumor-associated macrophages have been reported to be highly suppressive of lymphocyte functions (40), but may themselves be tumoricidal (41, 42). Thus, the effect of adherent cells on the generation of IL-2-activated EAL in our study was of interest. For these experiments, adherent cells were removed after Percoll gradient centrifugation by plastic adherence and the nonadherent lymphocytes were activated for 4 days with IL-2. As shown in Fig. 4, the removal of adherent cells prior to activation resulted in increased tumoricidal activity of all patients tested against both FMEX and autologous tumor cells, indicating that adherent cells may cause some suppression of the generation of tumoricidal activity of EAL.

**Phenotype of IL-2-activated EAL.** Previous studies have shown that TIL isolated from solid tumors were comprised largely of CD3+ cells (T-cells) (30). To assess the types of cells present in IL-2-activated EAL, surface markers associated with T-cells, monocytes, and NK cells were analyzed in specimens from four patients. These patients had a range of 36.4–65.6% CD3+ cells, 20–34.5% CD8+ cells, 11.1–35.0% CD16+ cells, 10.2–14.2% Leu 7+ cells, and 11.5–12.8% Leu19+ cells. Because a significant portion of the cells consisted of CD3+ cells, experiments were performed to determine if these cells were responsible for the cytolytic activity of EAL. To determine the phenotype of IL-2-activated EAL from effusions, two methods were used, i.e., affinity column separation of monoclonal antibody-labeled EAL and serological depletion studies. In Table 3, EAL were separated by affinity columns into CD3+ and CD3− populations and NKH-1+ and NKH-1− cells. NKH-1 (Leu 19) has been reported to correlate with LAK activity (43), although some cytotoxic T-cells also express this marker (44). As shown, cytotoxic activity against both FMEX and autologous fresh tumor cells was enriched in the CD3− and NKH-1+ populations, but some activity remained in the CD3+ and NKH-1− cells. These results suggest that IL-2-activated EAL were predominantly non-T-cells that were Leu 19−.

To further examine the phenotype of EAL, serological depletion of IL-2-activated cells was performed. As shown in Table 4, the removal of CD3+ cells by complement depletion slightly reduced cytotoxicity of EAL. This was similar to the results in Table 3. Removal of CD8+ and Leu M3+ cells also had minimal effect on cytotoxicity against both FMEX and autologous tumor cells. However, treatment of EAL with CD2, which removed both T-cells and NK cells, and CD16 (Leu 11), which removed primarily NK cells, eliminated much of the tumoricidal activity.

**Activation of Cytotoxicity in Autologous Effusion Fluid.** To determine whether ascites or effusion fluid would suppress the IL-2-induction of EAL, we performed parallel experiments using cultures of effusion cells incubated with IL-2-containing medium in the presence of 5% pooled human AB serum or 5% autologous effusion fluid. Fluid was collected prior to the addition of heparin to effusions and allowed to clot. Fig. 5 shows the results of the lysis of autologous tumor cells from two patients. There were not significant differences seen between the activation of EAL or whole effusion-associated cells in either human AB serum or effusion fluid. In the absence of IL-2, there was little cytotoxicity seen in either AB serum or effusion fluid, again demonstrating the requirement for IL-2.

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**Table 1. Lysis of autologous and heterologous fresh tumors by IL-2-activated EAL.**

<table>
<thead>
<tr>
<th>EAL: Patient</th>
<th>FMEX</th>
<th>Lysis of fresh tumors from: (%) specific lysis + SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 2</td>
<td>34.1 ± 2.1</td>
<td>10.5 ± 0.5, 6.7 ± 0.4, 28.8 ± 1.1</td>
</tr>
<tr>
<td>Patient 4</td>
<td>42.3 ± 1.3</td>
<td>13.9 ± 0.7, 7.7 ± 0.8, 10.6 ± 0.5</td>
</tr>
<tr>
<td>Patient 5</td>
<td>29.7 ± 1.5</td>
<td>21.2 ± 1.0, 16.2 ± 0.6, 20.7 ± 1.1</td>
</tr>
<tr>
<td>Patient 7</td>
<td>27.7 ± 1.0</td>
<td>15.9 ± 0.8, 7.7 ± 0.8, 10.6 ± 0.5</td>
</tr>
<tr>
<td>Patient 12</td>
<td>49.7 ± 2.2</td>
<td>10.6 ± 0.5, 20.7 ± 1.1, 10.6 ± 0.5</td>
</tr>
</tbody>
</table>

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Table 2. Lysis of autologous and heterologous fresh tumors by IL-2-activated EAL.

EAL obtained from the indicated patients were activated with IL-2 for 4 days and tested for their cytolytic activity against the indicated target cells. The effector/target (E:T) ratio of 20:1 is reported, and italicized numbers indicate lysis of autologous tumor target cells.
cells, containing both tumor cells and lymphocytes, could be activated by IL-2 to kill autologous tumor cells. Freshly isolated EAL either from ascites fluid or pleural effusions of patients with ovarian or metastatic carcinoma were incapable of lysing autologous tumor cells or FMEX tumor cells, similar to TIL from patients with solid tumors as reported by other investigators (23, 25–27). An earlier report by Uchida et al. (33) has indicated that fresh large granular lymphocytes isolated from pleural effusions could serve effectively against autologous tumor cells. In our hands, we were not able to detect significant activity until the EAL were triggered by IL-2, much like the observations with TIL. A second report by Allavena et al. (45) is similar to ours in that whole lymphocytes isolated from the ascites fluid of patients with ovarian carcinoma had little anti-tumor activity without pretreatment with IL-2. This latter study, however, made no attempt to identify the effector cells in the ascites fluid responding to IL-2.

Unlike TIL (26, 28, 29), the present study shows that the active cell population induced by IL-2 in EAL was predominantly CD3− NK/LAK cells with non-MHC-restricted lytic activity, although EAL consisted of approximately 36–66% CD3+ cells. Treatment of EAL with anti-CD3 antibodies plus complement did partially decrease cytotoxicity, indicating that some CD3+ MHC-unrestricted effector cells were also present that could be activated by IL-2. Longer incubation of EAL with IL-2 beyond 5 days may be required to generate and expand CD3+ EAL to a larger extent. Alternatively, monocytes may be required as accessory cells for the activation of CD3+ EAL, as has been shown for the activation of CD3+ LAK-type cells (46). Studies are underway to investigate these possibilities.

The initial inactivity of freshly isolated EAL may be due to...
sucessor factors or suppressor cells present in the effusions. Both types of suppressive mechanisms have been reported from solid tumors and effusions (23, 40, 47). Whatever the cause might be for the inactivity in freshly isolated EAL, it apparently could be overcome by IL-2. Macrophages found in the peripheral blood or solid tumors can act as suppressor cells against fresh NK cells and in the generation of LAK cells (39). Removal of monocytes prior to IL-2 activation did improve EAL generation in our hands, suggesting that some macrophage inhibition of EAL activation exists, similar to that of LAK generation (37, 38). We have earlier reported that IL-2 can generate LAK cells against autologous monocytes (35). It is tempting to speculate that local induction of EAL by IL-2 may be beneficial not only in boosting antitumor activity at the tumor site, but also in eliminating monocytes that serve as suppressor cells.

In addition to tumor-associated macrophage suppression, ovarian cancer ascites fluid has been reported to inhibit in vitro lymphocyte proliferation and NK cell function (47, 48). The present study indicates that IL-2 can overcome this suppression. Because EAL can be generated by IL-2 in autologous serum, in vitro instillation of IL-2 to locally activate antitumor activity may present an efficient means of immunotherapy for patients with ovarian carcinoma and metastatic pleural effusion. It is well documented, at least with chemotherapeutic agents, that the ratio of total drug exposure for the peritoneal cavity to that for the systemic circulation is 4–3000 times greater (49). This means that much less IL-2 may achieve significant efficacy with little toxicity in local i.p. or intrapleural instillations. The earlier results of Lotze et al. (50), indicating severe toxicity with 8–38 \times 10^7 U/kg i.p., have discouraged the use of this route for IL-2 therapy. However, it is possible that the optimal immunomodulatory dose of IL-2 may be much less than the maximum tolerated dose. Sondel et al. (51), using low-dose sustained i.v. IL-2 infusion, without LAK cell-adaptive therapy, have achieved some beneficial effects in patients with advanced renal cell carcinoma with acceptable toxicity. Combined i.v. low dose cyclophosphamide and low dose IL-2 therapy has also appeared to reduce toxicity with increased efficacy in remission of disseminated melanoma (52). In fact, lung carcinoma patients administered 1,000–15,000 units of IL-2/kg every 8 h for 48 h before their tumor was resected showed elevated NK/LAK activity not accompanied melanoma (52). In fact, lung carcinoma patients administered 1,000–15,000 units of IL-2/kg every 8 h for 48 h before their tumor was resected showed elevated NK/LAK activity not accompanied by tumor inhibition (53).

More importantly, a recent report by Yasumoto et al. (54) using high dose IL-2 will ensure lowered toxicity, leading to a more tolerable and effective immunotherapeutic regimen.


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