Lymphokine-activated Killer Cells: Lysis of Fresh Syngeneic Natural Killer-resistant Murine Tumor Cells by Lymphocytes Cultured in Interleukin 2

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ABSTRACT

Normal splenocytes that are cultured in the lymphokine, interleukin 2 (IL-2), for as short as 2 days develop lytic activity for fresh syngeneic natural killer-resistant tumor cells as well as natural killer-sensitive YAC cells in a 4-hr 51Cr release assay. Lymphokine-activated killer (LAK) cells do not lyse syngeneic fresh lymphocytes but do lyse syngeneic concanavalin A-induced lymphocyte blasts. Lysis is not due to the presence of lectin or xenogeneic serum and appears to be an intrinsic property of lymphocytes activated in IL-2. The activation appears universal in that lymphocytes from all strains of mice activated in this manner exhibited similar patterns of lysis for fresh tumor target cells.

To characterize the cells responsible for this lysis, we analyzed the phenotypic expression of surface markers on these cells with depletion techniques using monoclonal antibody and complement. These studies indicate that the precursor of the LAK cell is Thy-1+ and nonadherent to plastic or nylon wool. Lysis of syngeneic tumor was inhibited when LAK cells were cultured with an anti-Thy-1.2, or anti-Lyt-2.2 monoclonal antibody and complement but not with anti-Lyt-1.2 monoclonal antibody and complement, indicating that the observed lytic activity was due to a Thy-1+ Lyt-1−2+ cell. Furthermore, LAK cell-mediated lysis could be inhibited by the addition of anti-Lyt-2 or LFA-1 monoclonal antibody to cytotoxicity assays. Cold target inhibition analysis revealed that the syngeneic tumor cells were lysed by recognition of a determinant not present on normal lymphocytes or lymphocyte blasts. This lysis of fresh solid tumor cells by lymphoid cells grown in IL-2 may be of value in the study of tumor-host immunological interactions. The biological significance of tumor lysis by IL-2-activated cells requires further study.

INTRODUCTION

The ability to generate T-cells with lytic activity for fresh autologous tumor after specific in vitro sensitization has met with limited success in both murine and human studies. In an attempt to reproducibly generate T-cells with lytic activity to fresh autologous tumor, we have been studying the nonspecific activation of T-lymphoid cells caused by short-term culture in IL-2.

In previous reports from our laboratory, we have described optimal conditions for the production of IL-2 and long-term in vitro growth of both murine and human T-lymphoid cells (17, 27–32, 37). In human studies, the LAK cells may be induced with only a 2-day incubation of autologous lymphoid lymphocytes in the presence of IL-2. These cells expressed the phenotypic characteristics of allogeneic CTL based on their susceptibility to lysis by OKT-3 and OKT-8 monoclonal antibody and complement, and lysed a variety of fresh autologous human tumor cells as well as fresh allogeneic tumor cells, but not normal lymphocytes (8–10). Little information exists, however, as to the presence of LAK cells and their characterization in the mouse (37). In this paper, we report on the presence of LAK cells in a variety of mouse strains, the precursor and effector phenotypes of the murine LAK cells, and the specificity of lysis of these cells. The high levels of lysis by LAK cells of syngeneic NK-resistant tumor cells may be of value in studying interactions between lymphoid cells and tumors as well as for adoptive immunotherapy.

MATERIALS AND METHODS

Animals. C57BL/6, BALB/c, DBA/2, AKR, C3H, and B10.D2 mice were obtained from the Animal Production Colonies of the NIH, Bethesda, MD.

Tumors. Two sarcomas, MCA-102 and MCA-106, were induced by the injection of 0.1 ml of 1% 3-methylcholanthrene in sesame oil into the right hind limb of female C57BL/6 mice as described previously (24). The tumors were maintained by repeated passage in syngeneic hosts and used in these experiments in the fourth to seventh transplant generation. P-815 (H-2b) was passaged in ascites form in DBA/2 syngeneic mice, and EL-4 (H-2b) was passaged in ascites form in C57Bl/6 mice.

Preparation of Tumor Cell Suspensions. Tumor cell suspensions were prepared by short trypsinization as described previously (37). In brief, tumors were cut into 5-mm fragments in HBSS, washed once with 0.25% trypsin in Dulbecco’s phosphate-buffered saline without Ca++ and Mg++ (NIH Media Unit), and then trypsinized at room temperature for 10 min. The supernatant containing released cells was removed into an equal volume of cold RPMI Medium 1640 (Grand Island Biological Co., Grand Island, NY), penicillin (100 units/ml), and streptomycin (100 μg/ml). This medium is referred to in this paper as RPMI medium. An equal volume of fresh trypsin solution was added to the trypsinization flask, and the procedure was repeated once. The pooled cell suspension supernatants were washed 3 times with cold RPMI medium by centrifugation for 7 min at 100 × g.

In order to lyse erythrocytes, cell suspensions were treated with buffered ammonium chloride lysing solution.

Preparation of Splenocyte Suspensions. Spleens were removed aseptically into HBSS and were passed gently through a 40 mesh screen and through a single layer of 100 mesh nylon. The cells were centrifuged at 100 × g for 7 min, and the pellet was resuspended in a buffered ammonium chloride solution for 1 min at room temperature to lyse the RBC. After 1 min, the suspension was diluted in RPMI medium and washed 3 times. For the growth of T-cells, the final cell pellet was
suspended in complete RPMI Medium 1640, (Grand Island Biological Co.) supplemented with 0.03% fresh glutamine (NIH Media Unit), 1 µM sodium pyruvate (Microbiological Associates, Walkersville, MD), 0.1 mM nonessential amino acids (Microbiological Associates), 5 x 10^5 M 2-mercaptoethanol, penicillin (100 units/ml), streptomycin (100 µg/ml), and 10% heat-inactivated fetal calf serum (Grand Island Biological Co.).

Preparation of Con A-stimulated Blast Cells (Con A Blasts). Splenocytes (4 x 10^8) were incubated for 48 hr in 2 ml of CM containing 10 µg of Con A in the wells of Costar 24-well plates (Catalogue No. 3524; Costar, Cambridge, MA).

In Vitro Sensitization. Allogeneic in vitro sensitizations were performed using conventional techniques (27, 29, 30). In brief, spleen cells prepared as described above. Stimulator cells received 2000 rads in a γ-irradiator. Responder cells (4 x 10^8) and 10^8 irradiated stimulator cells were mixed and added to each well of a Costar 24-well tissue culture plate in 2 ml of CM. The cells were then incubated for 4 days at 37°, 5% CO2. In experiments where large numbers of alloreactive cells were required for antibody and complement depletion, 75 x 10^6 responder cells were admixed with 15 x 10^6 irradiated stimulator cells (2000 rads) in 90 ml of CM in 250 ml upright culture flasks (Falcon Plastics, Oxnard, CA). The cells were incubated at the culture conditions described previously.

Preparation of IL-2. IL-2, free of lectin, was prepared as described previously (31). In brief, 2 x 10^8 BALB/c splenocytes were incubated in 50 ml of CM containing Con A (10 µg/ml) at 37°, 5% CO2 for 2 hr. The cells were then washed 3 times with HBSS, and the pellet was resuspended in 50 ml of CM. Two ml of the washed cell suspension was placed into individual wells of 24-well Costar tissue culture plates and incubated for an additional 24 hr. The supernatant was collected and treated as described previously. IL-2 prepared in this manner was greater than 95% Con A-free (31). Highly purified human IL-2 produced by the high producer Jurkat clone and purified by passage over an anti-IL-2 affinity column was kindly supplied by Dr. P. Robb, E. I. duPont, Glendale, PA.

Growth and Activation of LAK. For the activation of LAK cells, splenocytes were prepared at a concentration of 4 x 10^6 viable cells/ml. One ml of the cell suspensions and 1 ml of IL-2 were added to each well of a Costar tissue culture plate. After 2 to 7 days at 37° with 5% CO2, cells were harvested, counted in 0.25% trypan blue, and resuspended either in complete medium or in RPMI medium for cytotoxicity testing. Splenocytes used for effector and precursor cell experiments were lymphokine-activated for 3 to 4 days at a concentration of 2 x 10^6 cells/ml in either 50% IL-2 or at various dilutions of Jurkat IL-2. Cells were cultured in a final volume of 2 ml in 24-well Costar plates, 18 to 20 ml in 30-ml supine culture flasks, or in 150 ml in 750-ml supine culture flasks.

Depletion with Antibody and Complement. Fresh C57BL/6 lymphocytes were adjusted to a concentration of 10^7 cells/ml in cytototoxicity medium composed of RPMI 1640, 0.3% bovine serum albumin, and 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, containing monoclonal antibody at the desired final concentration. Anti-Thy-1.2, anti-Lyt-2.2, and Lyt-1.2 were used at a 1:500, 1:100, and 1:200 dilutions, respectively (New England Nuclear, Boston, MA). Cells were incubated for 30 min at 37° with intermittent agitation, were centrifuged, and were resuspended in one-half of the original volume in a 1:5 to 1:6 dilution of lowtox rabbit complement (Cedarlane, Hicksville, NY) for an additional 60 min at 37°. Cells were washed 3 times in HBSS and resuspended in CM for further study. LAK cells or in vitro-sensitized cells were passed over a Lymphocyte-M (LSM; Lederle Laboratories, Ontario, Canada) gradient to remove dead cells and debris prior to depletion. All other steps were performed as described above.

Chromium Release and Cold Target-Cell Inhibition Assays. A 4-hr chromium release assay was performed as described previously (30). For the cold target-cell inhibition assay, nonradioactive (cold) blocking cells were tested for their ability to inhibit the lysis of MCA-102 tumor targets by cytotoxic T-cells; 50 µl of the cold blockers at the desired concentration were incubated with 50 µl of effector cells in wells of microtiter plate (Catalogue No. 76-311-05; Linbro Chemical Co., Hamden, CT) for 30 min at 37°; 100 µl of the ^51Cr-labeled MCA-102 tumor target cells were then added to the wells. The plates were incubated for an additional 4 hr prior to harvest as described for the ^51Cr release assay.

Role of Lyt-2 and LFA-1 in LAK Effector Cell Lysis. The effect of anti-Lyt-2 and anti-LFA-1 monoclonal antibodies on cytotoxicity of CTL and LAK was studied by incubating 5 x 10^6 effector cells in microtiter plates with various dilutions of the following monoclonal antibodies: (a) anti-Lyt-2 53.6.7 (15), concentrated 10 times; and (b) anti-LFA-1 H35-89.9 (25) ammonium sulfate precipitate from ascites, kindly supplied by Dr. P. Goisteen and Dr. M. Pierres, Centre d'Immunologie, France. After 30-min incubation at 37° in 150 µl of medium, 5 x 10^5 ^51Cr-labeled target cells were added in 50 µl. Plates were spun for 5 min at 500 rpm and incubated for 5 hr at 37°. Assays were harvested as described above.

Adherent Cell Depletion. Splenocytes were incubated at 2 x 10^6 cells/ml in 45 ml of CM in supine 650-ml tissue culture flask (Costar) at 37° for 1 hr. Nonadherent cells were removed and incubated for 1 hr at 37° in a 50-ml centrifuge tube containing 0.8 g nylon wool, and the nonadherent cells were harvested and resuspended in CM.

RESULTS

Cytotoxic Activity and Specificity of LAK. Normal fresh C57BL/6 lymphocytes or lymphocytes cultured for 5 days in CM or IL-2 were tested for lytic activity versus several normal and tumor targets in a 4-hr chromium release assay (Table 1). Fresh C57BL/6 lymphocytes or lymphocytes cultured in complete medium for 5 days demonstrated 20 and 8% lysis, respectively, for the YAC, NK-sensitive target, but not for any of the other targets tested. Lymphocytes activated in IL-2 for 5 days manifested significant lysis for the fresh, NK-resistant, syngeneic MCA-102, MCA-106, and EL-4 lymphoma as well as the YAC target. No lysis of fresh C57BL/6 lymphocytes was observed although good cytotoxicity was found against C57BL/6 syngeneic Con A blasts.

These results indicated that IL-2 will activate fresh lymphocytes to kill NK-resistant tumor and blast target cells.

Kinetics of LAK Activation. Normal C57BL/6 lymphocytes were activated in IL-2 or CM and tested for in vitro cytotoxicity at 100:1 effector:target ratios versus 3 syngeneic fresh-tumor target as well as C57BL/6 fresh lymphocytes and Con A blasts in a 4-hr chromium release assay (Chart 1). Appreciable lytic activity for tumor targets was observed by 2-day culture in IL-2 with peak reactivity on Day 4 of culture. LAK cells at no time lysed fresh C57BL/6 lymphocyte; however, substantial lysis was seen for Con A blasts. Fresh C57BL/6 lymphocyte or lymphocytes cultured in CM for up to 7 days at no time demonstrated significant lysis for any of the targets tested.

IL-2 as Stimulus for the Generation of LAK Cells. The lytic capacity of cells cultured in supernatants containing IL-2 was due only to IL-2 and not Con A, fetal calf serum, or other

<table>
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<th>Target</th>
<th>Fresh C57BL/6 cultured in medium</th>
<th>C57BL/6 cultured in IL-2</th>
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<tr>
<td>MCA-102</td>
<td>1 ± 1</td>
<td>53 ± 2</td>
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<tr>
<td>MCA-106</td>
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<tr>
<td>EL-4</td>
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<td>43 ± 2</td>
</tr>
<tr>
<td>C57BL/6 lymphocyte</td>
<td>-12 ± 3</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>C57BL/6 Con A blast</td>
<td>3 ± 2</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>YAC</td>
<td>20 ± 1</td>
<td>72 ± 1</td>
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</table>

* Effector:target ratio, 100:1.  
** Mean ± S.E.
lymphokines present in IL-2 preparations. In 2 separate experiments, C57BL/6 lymphocytes cultured in IL-2 produced in 1% mouse serum, and that never were in contact with fetal calf serum, generated LAK cells capable of lysing the MCA-102 tumor in a 4-hr chromium release assay (Table 2). Furthermore, highly purified, homogeneous Jurkat IL-2, free of other lymphokine activity, was also capable of activating LAK cells at a 1:12,500 or 1:25,000 dilution (Table 3). Similar levels of lysis were achieved when C57BL/6 lymphocytes were activated in highly purified IL-2 produced by the EL-4 high producer tumor cell line (provided by Dr. Alfred Chang; data not shown).

Furthermore, lysis of syngeneic tumor was not effected by extensive washing of effector cells after culture in IL-2 or by conducting the lytic assay in α-methylmannoside (20 μg/ml; data not shown).

Thus, it appears that the lytic activity of splenocytes that results from activation in IL-2 is not dependent on exposure to either Con A, xenogeneic serum, or lymphokines other than IL-2.

**Lymphocytes from Multiple Strains of Mice and Capability to Generate LAK.** In order to determine if the activation of LAK cells was a strain-specific phenomenon, lymphocytes from multiple mouse strains were cultured in IL-2 or CM for 5 days and tested for lytic activity versus both the fresh MCA-102 and the EL-4 tumor target (Chart 2). Splenocytes from 6 of 6 strains tested were capable of generating substantial lysis for both the MCA-102 and EL-4 tumor targets. Cells cultured in CM exhibited minimal or no lysis for the EL-4 and MCA-102 target cells.

**Cold-Target andBlocking Studies.** In contrast to the significant levels of lysis of the syngeneic 102 tumor and C57BL/6 Con A blasts, normal syngeneic splenocytes were poorly lysed by C57BL/6 cells grown in IL-2. To determine the effects of differences in cell lysis that were due to intrinsic differences in lysability, DBA/2 splenocytes that were sensitized in vitro to C57BL/6 cells were used as effectors against several C57BL/6 target cells. Both MCA-102 and Con A blast cells were more sensitive to lysis by the in vitro allosensitized cells than were fresh splenocytes (data not shown).

Since C57BL/6 splenocytes were not as sensitive to lysis as the tumor target cells, cold-target inhibition studies were performed to determine whether these cells shared a common specificity that could not be detected using lysis in the 51Cr release assay. Nonlabeled splenic C57BL/6 or unlabeled Con A blast cells were mixed with the effector cells and assayed at an effector:target cell ratio of 10:1 on 51Cr-labeled MCA-102 target cells using effector cells grown in IL-2. As shown in Chart 3, both syngeneic normal splenocytes and syngeneic Con-A blasts resulted in no inhibition of the lysis of syngeneic MCA-102 tumor even at high blocker:effector cell ratios. Cold MCA-102 cells, however, caused strong inhibition of lysis which was directly proportional to the blocker:effector cell ratios. Similar results were obtained in 5 experiments. These data suggested that the cells activated in IL-2 recognized a determinant on the MCA-102 cell surface with either limited or no expression on the normal splenocyte cell surface and distinct from the determinant that resulted in lysis of C57BL/6 Con A blast cells.

Cold target inhibition studies of the lysis of C57BL/6 Con A blast cells revealed that both fresh 102-tumor cells and Con A blast cells were capable of completely inhibiting lysis (Chart 4).
Tumor Lysis by Cells in IL-2

Thus, although both syngeneic MCA-102 and Con A blasts are lysed by cells grown in IL-2, different determinants are recognized on these 2 cells. The tumor cell contains the determinant recognized on the blast cells but contains, as well, a different determinant not present on the blast cells.

The Precursor Cells of LAK are Nonadherent and Thy-1.2-Positive. Normal C57BL/6 lymphocytes received no treatment or were depleted of adherent cells by incubation on plastic Petri dishes and passage over nylon wool. Comparable levels of lytic activity were generated when intact or adherence-depleted cells were cultured in IL-2 for 5 days and tested against the syngeneic MCA-102 or EL-4 tumor target (Table 4), however, no lysis was detected against fresh C57BL/6 lymphocytes. Intact or adherence-depleted cells demonstrated no detectable level of lytic activity when cultured in CM for 5 days.

Normal C57BL/6 splenocytes received no treatment, treatment with rabbit complement, or treatment with a monoclonal anti-Thy-1.2 antibody and complement. In 2 of 2 experiments, comparable levels of lysis were obtained when responder C57BL/6 lymphocytes were treated with complement or not (Table 5). The ability to generate allospecific cytotoxicity from
Thy-1.2-depleted cultures was totally eliminated whether the adherence-depleted cells was treated in the same manner. LAK cells generated 49% killing. To determine what role Lyt-2 and LFA-1 membrane molecules played in LAK cell lysis, LAK cells or allogeneic CTL were pretreated with monoclonal antibody directed against either the Lyt-2 or LFA-1 membrane molecule prior to being used as effector cells in a chromium release assay (Table 7). BALB/c anti-C57BL/6 and C57BL/6 anti-BALB/c in vitro-sensitized effector cell populations generated 23 and 75% lysis for the respective EL-4 and P-815 allogeneic tumor targets at a 20:1 effector:target ratio in a 5-hr chromium release assay. Addition of anti-Lyt-2 or anti-LFA-1 monoclonal antibody to anti-EL4 allogeneic CTL totally inhibited lysis of EL-4 at concentrations as low as 1:200 and 1:100,000, respectively. Anti H-2b (anti-BALB/c) allogeneic effector cells demonstrated a similar inhibition of lytic activity when treated in the same manner. LAK cells generated 49% lysis for the syngeneic EL-4 target and 42% lysis for the allogeneic P-815 target at a 20:1 effector:target ratio. Lytic activity against the syngeneic EL-4 target was inhibited by 57% by the Lyt-2 reagent and completely inhibited by the LFA-1 reagent at a 1:200 and 1:100,000 dilution, respectively. A similar pattern of inhibition was demonstrated for the allogeneic P-815 target. Both anti-Lyt-2 and LFA-1 had no effect on the spontaneous release of the P-815 target, but slightly increased the spontaneous release of the EL-4 target to -16 and -18%, respectively. Thus, membrane molecules essential to LAK cell lysis reacted with anti-LFA-1 antisera and, to a lesser extent, anti-Lyt-2 antisera.

**DISCUSSION**

The development of techniques for the generation of T-lymphocytes with cytotoxic reactivity for fresh autologous tumor cells has been an elusive goal of many laboratories in recent years. Our laboratory has recently reported that the incubation of either normal or cancer patients' lymphocytes in IL-2, for as short as 48 hr leads to the generation of significant lytic activity for autologous or allogeneic fresh solid tumor (6). Human LAK cells are capable of lysing a wide variety of histologically distinct NK-resistant solid tumor cells, but do not lyse autologous peripheral blood lymphocytes, Con A lymphoblasts, or single-cell suspension from several normal adult tissues (8-10). The precursor of the human LAK cell is distinct from both NK and T cells based on serological phenotype, density gradient sedimentation, and tissue distribution, while the human LAK effector cells bears all the phenotypic, morphological, and functional characteristics of CTL (10). The biological role of these LAK cells in the human is unknown. We have developed an animal model of the
Tumor Lysis by Cells in IL-2

Table 6

<table>
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<tr>
<th>Treatment</th>
<th>Effector:targ</th>
<th>% of lysis^a</th>
<th>Allogeneic^b</th>
<th>LAKc</th>
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<th>LAK</th>
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<td>39.1 ± 1.0</td>
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<td>Complement</td>
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^a 4-hr chromium release assay.
^b 5 x 10^6 P-815 (H-2^d) target.
^c 5 x 10^6 MCA-102 (H-2^d) target.
^d Mean ± S.E.

Table 7

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<tr>
<th>Effector</th>
<th>Treatment</th>
<th>EL-4 % of lysis^a</th>
<th>P-815 % of lysis^a</th>
<th>% of inhibition</th>
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^b Mean ± S.E.

LAK phenomenon to aid in studies of the role of LAK cells in vivo.

In this report, we have demonstrated that normal nonsensitized murine splenocytes cultured in IL-2 for as short as 2 days developed lytic activity for fresh NK-resistant syngeneic tumor and autologous Con A blasts, but not normal lymphocytes. This reactivity was maximal after 4 days of culture of lymphocytes in IL-2. LAK cells displayed broad lytic reactivity for syngeneic and allogeneic tumor or Con A blast targets. Furthermore, activation of LAK cells appears to be a general phenomenon in murine strains, since all strains tested (Chart 2) were capable of generating lysis for LAK-sensitive, NK-resistant tumor targets upon IL-2 stimulation.

The activation of LAK cells to cause lysis of syngeneic tumor was not dependent on Con A or lymphokine other than IL-2 present in crude supernatants, since lymphocytes cultured in highly purified human- or mouse-derived IL-2 were also lytic for tumor. Furthermore, we^3 and others (1) have been unable to induce lytic activity by exposing C57BL/6 lymphocytes to Con A under a wide variety of culture conditions in the absence of exogenous IL-2. No differences in the lytic activity of LAK cells were observed when the cytotoxicity assay was performed in a-methylmannoside, indicating no dependence on lectin in the target cell recognition by LAK cells.

Several investigators have reported the nonspecific activation of T-lymphoid cells by fetal calf serum determinants (16, 21, 36).

^3 A. Mazumder, M. Rosenstein, and S. A. Rosenberg, submitted for publication.
Although fetal calf serum is often present in our IL-2 preparation, LAK activation is not dependent on the presence of xenogeneic serum. Lymphoid cells cultured in IL-2 produced in 1% mouse serum exhibited lysis similar to that of LAK activated in fetal calf serum containing IL-2 preparations. In each of our experiments, control cultures of splenocytes incubated in CM containing fetal calf serum were included, and in no instance was significant lytic activity generated.

We have used monoclonal antibody and complement depletions techniques to characterize the cell surface phenotype of cells activated in IL-2. The LAK effector cell is sensitive to treatment with anti-Thy-1.2 or anti-Lyt-2.2 monoclonal antibody and complement, but only slightly sensitive to anti-Lyt-1.2 antibody and complement. NK cells are thought to express Lyt-1 and low levels of Thy-1 (6, 13), whereas conventional CTLs express high levels of Thy-1 and Lyt-2 but not Lyt-1 on their cell surfaces (34, 35). As demonstrated above, the LAK cell expressed a conventional CTL phenotype (Thy-1+ Lyt-1-2+) and, therefore, appears distinct from NK cells. Another important difference between LAK cells and NK cells is the ability of LAK cells to kill a variety of fresh tumor target cells that are resistant to NK cell lysis. These findings are similar to those we have reported in the human in which the LAK cell is sensitive to OKT-3 and OKT-8, but not to OKT-4 antibodies (6), and also lysed NK-resistant fresh human tumor cells.

Further evidence that the murine LAK cell expresses the characteristics of CTL and not NK cells was the finding that both Lyt-2 and LFA-1 membrane antigens are involved in the cytolytic activity of LAK effector cells. Treatment of LAK cells or allogeneic CTL with anti-Lyt-2 or LFA-1 monoclonal antibody during cytolysis assays inhibited killing of the relevant syngeneic or allogeneic tumor target in a dose-dependent fashion. Reports by other laboratories (7) demonstrated no effect of anti-Lyt-2 on the blocking of NK activity, although concentrations of anti-LFA-1 in excess of those used in this report inhibited NK lytic activity by approximately 50%.

The precursor of the LAK cell appears to be a nonadherent Thy-1+ cell. This result is distinct from LAK generated in the human system (8–10), where no marker for the LAK precursor has yet been detected. While the lineage of the LAK cell in murine and human systems may be different, it is more likely that human and anti-T-cell reagents recognize determinants with different characteristics than those recognized by anti-Thy-1 antibodies in the mouse. Preliminary experiments with anti-Lyt-1.2 or Lyt-2.2 inhibits the generation of LAK. Experiments are currently in progress to determine if this is due to the elimination of a single precursor subset or if LAK activation requires the interaction of various subpopulations of lymphoid cells.

Activated lymphoid cells appear to acquire the capacity to lyse a wide variety of target cells depending on the activating stimulus. Paciucci et al. (22) and Zanling et al. (38) have reported that murine and human cells activated by alloantigens are capable of killing autologous tumor cells. Bonavida and Bradley (1), however, claimed that murine cells activated by alloantigens can lyse syngeneic tumor only in the presence of mitogen and that these cells appear to be identical to the cells lytic for alloantigens. Mazumder et al. (3) have recently shown that splenocytes from selected mouse strains can lyse fresh autologous NK-resistant tumor when incubated for 3 days with Con A, and that their lytic activity did not require the presence of Con A in the cytotoxicity assay. This phenomenon may be mediated through a common IL-2 activation pathway, since Lyt-1+ cells are required to generate this highly lytic cell type.

Other phenomena for the activation of cells lytic for tumor have been described such as natural killing (11, 14, 26), natural cytotoxicity (23, 33), activated cell killing (18, 19), activation by serum determinants (16, 21, 36, 39), 2-mercaptoethanol (12), tumor necrosis serum (2), polyinosinic acid (3), allogeneic stimulation, or specific mixed-lymphocyte tumor cell reactions (4, 5, 20, 22). In most of these studies, lytic activity was tested against cultured instead of the fresh tumor target cells used in our studies.

Of interest is the finding that T-cells activated in culture by IL-2 do not lyse fresh lymphoid cells. Since fresh lymphocytes are less lysable than tumor cells, we have attempted to use cold-target inhibition studies to determine if fresh lymphocytes share recognition determinants with tumor cells that are lysed by LAK. Although tumor cells, by nature of their larger size, are more efficient cold-target inhibitors, we have never seen inhibition of MCA-102 killing at even very high numbers of unlabeled fresh lymphocytes or Con A blast cells, suggesting, therefore, that a component on the MCA-102 cell surface that is recognized by LAK is absent or poorly expressed on the normal splenocyte cell surface. The surprising observation that Con A blast targets are lysed by LAK, but do not block LAK killing of MCA-102 at high cold-target inhibitor ratios, possibly reflects the heterogeneous nature of the LAK cell population. Cloning of the LAK cell population will further unravel this phenomenon.

The lysis of fresh tumor cells by syngeneic lymphoid cells incubated in IL-2 was an unexpected finding and is not well understood. The high lysis of syngeneic solid tumor cells that we have seen using a short-term cytotoxicity assay raises the possibility that these cells may be useful in in vivo studies of immune lysis by cytotoxic lymphoid cells. We have previously demonstrated that human cells grown in IL-2 exhibited lysis of autologous tumor and that mouse and human cells grown in IL-2 can be safely rein fused into syngeneic or autologous hosts. The potential immunotherapeutic effect of cells expanded in IL-2 in mouse tumor models is currently under investigation.

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Lymphokine-activated Killer Cells: Lysis of Fresh Syngeneic Natural Killer-resistant Murine Tumor Cells by Lymphocytes Cultured in Interleukin 2

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