SUCCESSFUL IMMUNOTHERAPY OF NATURAL KILLER-RESISTANT ESTABLISHED PULMONARY MELANOMA METASTASES BY THE INTRAVENOUS ADOPTIVE TRANSFER OF SYNGENEIC LYMPHOCYTES ACTIVATED IN VITRO BY INTERLEUKIN 2

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In several murine tumor models the adoptive transfer of sensitized lymphoid cells is capable of mediating the regression of established tumor (1–3). Identification of the most appropriate cells for use in adoptive transfer is a major problem. While cells specifically sensitized in vivo or in vitro to unique cancer antigens can be highly effective, it is often difficult to obtain these cells because of the poor immunogenicity of many murine tumors. This problem is especially cogent in considering human tumors in which poor immunogenicity and problems with availability of suitable preparations of human tumor cells for in vitro sensitization also exist.

In both mice and humans we have extensively studied, in vitro, the activity and specificity of activated killer cells. These cells can be generated in vitro by exposure of normal lymphocytes to interleukin 2 (IL-2)[1] (4, 5), lectins (6, 7), or pooled alloantigens (8). These activated cells recognize and lyse fresh autologous (in the human) or syngeneic (in the mouse) natural killer (NK)-resistant cancer cells, but do not lyse normal cells. The serologic phenotype of the precursor and effector cells of these activated killer cells has been extensively studied in mouse and man (4, 7, 8). The biologic role of activated killer cells is unknown, though it is known they can lyse tumor cells in Winn assays (9–11). However, because of the ease of generation of these cells and their potential applicability to the treatment of human tumors we have explored the possible use of these cells, adoptively transferred, in the treatment of a highly metastatic and virulent murine tumor. This report is the first demonstration that lymphokine-activated killer cells (LAK cells), activated in vitro by IL-2, can inhibit the growth of established melanoma pulmonary metastases. The kinetics of this phenomenon, the nature of the cells involved, and the in vitro activation stimulus required have been studied.

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[1] *Abbreviations used in this paper:* C′, complement; CM, complete medium; E, erythrocyte; HBSS, Hanks' Balanced Salt Solution; IL-2, interleukin 2; LAK, lymphokine-activated killer cells; NK, natural killer cells; PMA, phorbol 12-myristate-13-acetate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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Materials and Methods

Animals. C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and the animal production facilities of the National Institutes of Health and used in experiments when 12 wk or older.

Splenocytes. Spleens were removed aseptically and crushed with the hub of a syringe in complete medium (CM), which consisted of RPMI 1640 (Biofluids, Rockville, MD) with 0.1 mM nonessential amino acids and 1 μm sodium pyruvate (Microbiological Associates, Walkersville, MD), 5×10^{-5}M 2-mercaptoethanol, 100 μg/ml streptomycin, 100 U/ml penicillin 0.03% glutamine (NIH Media Unit), and 10% heat-inactivated fetal calf serum (Gibco Laboratories, Grand Island, NY). The cell suspension and spleen fragments were passed through a single layer of 10-gauge nylon mesh and the erythrocytes (E) lysed osmotically with buffered ammonium chloride solution at room temperature for 2 min. The cells were then centrifuged and washed two times with Hanks' Balanced Salt Solution (HBSS, Gibco). Fresh splenocytes prepared in this way were used for LAK cell production or as control effector cells. In the experiments with spleens from tumor-bearing mice (see Fig. 4), splenocytes harvested from mice injected with 10^5 B16 cells 2 wk previously were used for LAK cell production.

IL-2 Preparations. IL-2 production by concanavalin A pulsing (Con A pulse IL-2) of murine splenocytes has been previously described (12). Briefly, BALB/c splenocytes were incubated for 2 h with 10 μg/ml Con A (Miles Laboratories, Elkhart, IN), then washed three times in HBSS and resuspended in CM for 24 h. Supernatants were collected by centrifugation, passed through 0.45-μm filters (Millipore Corp., Bedford, MA), and refrigerated until use.

EL-4 IL-2 was produced as described previously (13). An EL-4 thymoma line (gift of J. Farrar, NIH) was maintained in RPMI 1640 with 3% fetal calf serum and 0.03% glutamine. Cells were washed twice in HBSS and placed at 10^6 cells/ml in RPMI 1640 with 0.03% glutamine, 50 μg/ml gentamicin, and 10 ng/ml phorbol 12-myristate-13-acetate (PMA) without serum for 48 h at 37°C. Collected supernatants were concentrated 100-fold using a Pellicon Millipore concentrator with a 10,000-dalton exclusion limit (Millipore), filtered (0.45 μm filter, Nalgene Labware, Rochester, NY), precipitated between 50% and 75% ammonium sulfate, and dialyzed as described previously (14).

Pure human IL-2 from Jurkat cells was kindly supplied by Dr. Richard Robb (Dupont Co.) and was prepared as reported elsewhere (15). Purity of the IL-2 was assessed by both two-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and sequencing the NH_2-terminal peptide. Jurkat IL-2 was diluted in CM before use. The dilutions used for LAK generation of Con A pulse IL-2, EL-4 IL-2, and Jurkat IL-2 were 1:2, 1:20, and 1:5,000, respectively.

Lymphokine Activation. Fresh C57BL/6 splenocytes were placed into 175 cm^2 (750 ml) flasks (Falcon Labware, Oxnard, CA) laid supine, in 175 ml of CM containing the optimal dilution of the particular IL-2 used, at a cell concentration of 2.5×10^6/ml. No IL-2 was added to control flasks. The flasks were incubated at 37°C, 5% CO_2 for 72–96 h. The cells were then harvested into sterile 250-ml centrifuge tubes (Falcon), washed three times with HBSS, and finally resuspended in HBSS for intravenous injection. Before each injection aliquots of the LAK or control cells were taken and tested for cytotoxicity in vitro.

In Vitro Cytotoxicity Assay. 18-h ^{51}Cr release assays were used as described previously (16). Briefly, tumor target cells were labeled with 400 μCi of Na ^{51}Cr assay (Amersham/Searle, Arlington Heights, IL) for 120 min in 0.5 ml of CM. They were then washed three times with CM and added at 5×10^3 cells/well to various numbers of the effector cells in round-bottomed microtiter plates (Linbro Scientific Co., Hamden, CT). The plates were incubated for 18 h at 37°C in 5% CO_2 and the culture supernatants then harvested with the Skatron Titertek System (Skatron A. S. Lierbyen, Norway) and counted in a gamma counter. Maximum isotope release was measured by incubation of the targets in with 0.1 N HCl. Spontaneous release was measured by incubation of the targets in CM

alone. The percentage of lysis was calculated by:

$$\% \text{ lysis} = \frac{\text{Experimental cpm} - \text{spontaneous cpm}}{\text{Maximal cpm} - \text{spontaneous cpm}} \times 100\%.$$

All determinations were made in triplicate and data are reported as the mean ± standard error of the mean at an effector to target ratio of 10:1, unless otherwise specified.

In Vivo Assay of Adoptive Immunotherapy. Cryopreserved cells from the B16 melanoma tumor line of C57BL/6 origin (17) were thawed and placed into tissue culture in CM for 12–18 h. The monolayer was then treated with trypsin and the cells obtained washed once with CM and then washed twice and resuspended with HBSS. Between 1×10^5 and 2×10^5 B16 tumor cells were injected into the tail veins of C57BL/6 mice that were greater than 12 wk old. In preliminary experiments (data not shown), pulmonary tumor nodules were found to be established within 3 d after injection (visually and histologically). LAK or control cells (usually 1×10^8 in 1 ml HBSS) were then infused into the tail vein at the times after tumor rejection mentioned in the experiments. On day 15 after tumor induction, the animals were sacrificed, the lungs harvested, and metastatic pulmonary nodules counted in a blinded fashion without knowledge of the treatment of that mouse. The complete enumeration of the metastases is possible because they formed black nodules on the surface of the lungs, as confirmed by histology.

In the spontaneous metastasis model shown in Fig. 1, 10^6 B16 tumor cells were injected into the right hind footpad and allowed to metastasize spontaneously to the lungs. Numerous pulmonary nodules were established by the time the primary tumors were ~1 cm in diameter. The right hind limb containing the primary tumor was then amputated and LAK cells were administered intravenously 3 d later. 15 d after the amputation, the

FIGURE 1. Representative lungs of a mouse from the group given no treatment (left) or LAK cells (right) in the experiment described in Table I.

lungs were harvested and the metastases counted as above.

When mentioned, 50 mg/kg or 150 mg/kg of cyclophosphamide (cytoxan) was infused intravenously 5–6 h before the transfer of the LAK or control cells.

For the survival studies shown in Fig. 3, 1×10^8 LAK cells or fresh splenocytes were administered on days 3, 5, and 7 after tumor induction and the mice followed daily for survival.

p values were obtained by analysis using standard *t*-test statistics.

Cell Surface Phenotype Determination. All depletion experiments were performed in cytotoxicity medium composed of RPMI 1640, 0.3% bovine serum albumin (Pathocyte 5; Miles Laboratories, Elkhart, IN), and 25 mM Hepes buffer. Thy-1.2$^+$ cells were removed by treatment with newborn rabbit complement (C′) plus an anti–Thy-1.2 monoclonal antibody (New England Nuclear, Boston, MA). Lymphocyte suspensions were adjusted to a concentration of 10^7 cells/ml in cytotoxicity medium containing the optimal concentration of antibody (1:200), and incubated in the cytotoxicity medium for 30 min at 37°C with intermittent agitation. The cells were then centrifuged and resuspended in one-half the original volume with a 1:5 dilution of low toxicity complement (Cedar Lane, Bethesda, MD). Incubation was continued for an additional 60 min at 37°C and the cells were then washed three times in HBSS and resuspended in CM for further study. LAK cells treated with C′ alone or with neither C′ nor antibody were included as controls. Treatments with antibody plus complement were done either before or after lymphokine activation. In vitro cytotoxicity was assayed before adoptive transfer.

Results

LAK Cells Exert Antitumor Effects In Vivo. C57BL/6 mice, injected intravenously with B16 tumor cells, were given LAK cells, fresh splenocytes, or no treatment on day 3 of tumor growth and the effects on metastasis development assayed on day 15. The cytotoxicity of the LAK cells infused was 72 ± 3%, while that of the fresh splenocytes was 5 ± 4%. Table I shows the number of metastases counted in each group, in a blinded fashion. The group given LAK cells intravenously had fewer lung metastases (mean of 14) than the control groups (means of 83 and 87 for the mice given no treatment and fresh splenocytes, respectively; *p* value < 0.01) (Fig. 1). Fresh splenocytes had no antimetastatic effect compared with the group given no therapy (*p* value > 0.5). Thus, the

TABLE I

In Vivo Antitumor Effect of LAK Cells

Cell transferred*	None	Fresh splenocytes	LAK cells
	No. of metastases‡		
	142	121	15
	101	108	12
	81	161	12
	64	34	3
	101	51	18
	45	73	22
	49	59	—
Mean	83	87	14

* 3 d after tumor induction by systemic administration of 2×10^5 B16 cells, C57BL/6 mice were given no therapy or infused intravenously with 1×10^8 syngeneic fresh splenocytes or LAK cells.

‡ 15 d after tumor induction, the lungs were harvested and the metastases counted blindly, as described in the text.

adoptive transfer of LAK cells can significantly decrease pulmonary B16 melanoma metastases.

Treatment of Spontaneous Metastases by Transfer of LAK Cells. The effect of intravenous administration of LAK cells on B16 tumor nodules in the lung that had spontaneously metastasized from a large primary in the right hind footpad was investigated. Fig. 2 illustrates that the infusion of LAK cells 3 d after amputation of the right hind limb caused a significant decrease ($p < 0.01$) in the number of spontaneous lung metastases counted on day 15 compared with mice given no treatment (4 ± 2 vs. 22 ± 3 nodules). The LAK cells were cytotoxic in vitro (72 ± 4% lysis). Thus, the adoptive transfer of LAK cells can also cause a significant decrease in spontaneous B16 melanoma lung metastases.

Transfer of LAK Cells Can Increase Survival Time. We wished to determine whether the marked decrease in number of metastases caused by the intravenous administration of LAK cells led to an improvement in survival. Because of the reported requirement for immunosuppression in other mouse models of adoptive immunotherapy we performed this experiment in mice pretreated with 500 rads whole body irradiation (18). The infusion of LAK cells on days 3, 5, and 7 after tumor induction into C57BL/6 mice bearing B16 melanoma lung metastases significantly lengthened survival time (52 ± 1 d) compared with irradiated mice given no therapy (21 ± 1 d; $p < 0.01$) (see Fig. 3). Fresh splenocytes given on the same days had no effect on the survival time (27 ± 1 d, $p > 0.5$). The in vitro cytotoxicity of the LAK cells was 48 ± 4%, while that of the fresh cells was 5 ± 1%. Thus, the adoptive transfer of LAK cells leads to a significant survival advantage.

Optimization of Treatment with LAK Cells. In order to optimize treatment with LAK cells, we investigated the effects of LAK cell administration at various times

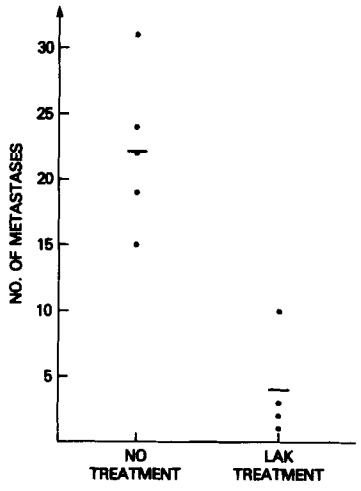

FIGURE 2. Decrease in spontaneously generated B16 pulmonary metastases caused by the intravenous adoptive transfer of LAK cells. B16 melanoma, injected into the right hind footpad, spontaneously metastasized to the lungs. 1×10^8 syngeneic LAK cells (generated using Con A pulse Il-2) were transferred intravenously 3 d after amputation of the involved limb and the number of lung nodules counted blindly 12 d later, as described in the text. Control animals were amputated but given no treatment.

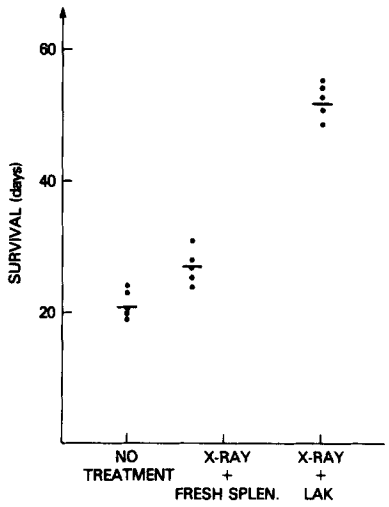

FIGURE 3. The adoptive transfer of LAK cells leads to a significant increase in survival time. C57BL/6 mice were irradiated with 500 rads and then induced intravenously with 1×10^5 B16 cells. On days 3, 5, and 7 of tumor growth, 1×10^8 LAK cells or fresh splenocytes were infused into two groups with another group receiving no therapy. The mice were then followed for length of survival.

after tumor induction with or without prior chemotherapy with cytoxan. It can be seen from Fig. 4 that LAK cells infused 7 or 11 d after tumor induction could exert significant antitumor effects ($p < 0.05$), although administration at 3 d after induction, was better ($p < 0.01$). It was also found that cytoxan infused alone or 5 to 6 h before the LAK cell infusions on day 3 had no effect on the in vivo efficacy of LAK cells (groups 5, 6, 7 vs. 8, 9, 10, respectively; $p > 0.5$). Again here, fresh splenocytes (group 3) had no in vivo antitumor effects.

We also wished to determine whether incubation with IL-2-containing supernatants was required for the generation of the in vivo effector cell, i.e., whether incubation in CM containing xenogeneic serum was sufficient. Fig. 4 demonstrates that splenocytes incubated in CM (group 4) alone had no antimetastatic effect in vivo or may have led to a slight increase in the number of metastases. The in vitro cytotoxicity of the LAK cells, fresh splenocytes, and splenocytes incubated in CM alone were 64 ± 4%, 3 ± 4%, and 7 ± 1%, respectively.

LAK Cells from Tumor-bearer Spleens Are Also Effective in Therapy. In order to investigate whether splenocytes from tumor-bearing animals could generate LAK cells capable of exerting antitumor effects in vivo, we incubated splenocytes from normal C57BL/6 mice or mice bearing 2-wk old B16 pulmonary tumors in Con A pulse IL-2 and administered those LAK cells to mice with B16 pulmonary metastases. We found that LAK cells from tumor-bearing animals were equivalent to those from normal animals (14 ± 2 and 15 ± 3 metastases, $p > 0.5$), in their ability to significantly decrease the number of lung metastases compared to control animals (52 ± 3; $p < 0.01$) (Fig. 5). The in vitro cytotoxicities of the LAK cells from normal and tumor-bearing mice were also equivalent (51 ± 4% and 48 ± 3%, respectively).

The In Vivo and In Vitro LAK Effector and Precursor Cell is Thy-1$^+$. The cell

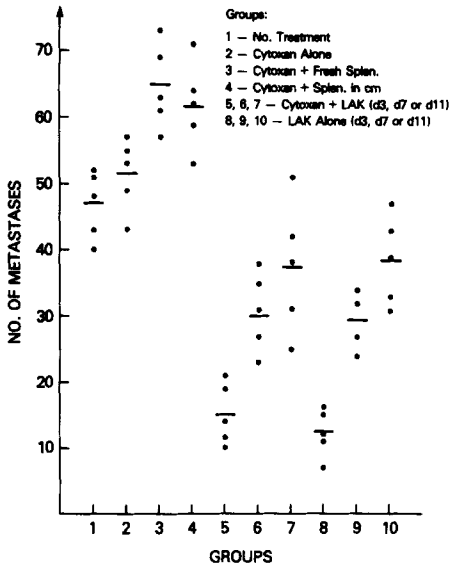

FIGURE 4. Kinetics and chemotherapy studies. C57BL/6 mice, induced with 2×10^5 B16 melanoma cells intravenously, were split into 10 groups as shown. Each mouse was individually weighed and 150 mg/kg cytoxan was infused intravenously alone or 6 h before the adoptive transfer, on day 3 after tumor induction, of 1×10^8 fresh splenocytes or 1×10^8 splenocytes incubated in CM alone or on days 3, 7, or 11 of 1×10^8 LAK cells generated by Con A pulse IL-2. Five mice received 1×10^8 LAK cells on days 3, 7, or 11 after tumor induction without prior cytoxan. On day 15 after tumor induction, the lungs were harvested and the metastases counted blindly, as described in the texts. Control animals were given no therapy.

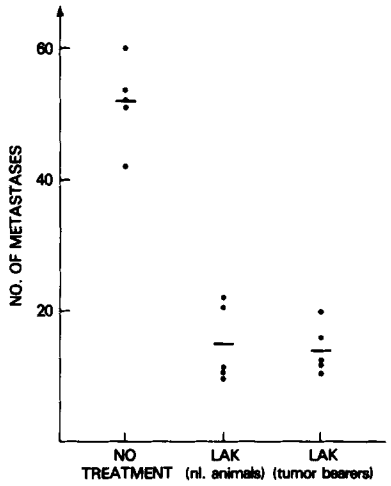

FIGURE 5. 1×10^8 LAK cells, generated by incubation in Con A pulse IL-2, from spleens of normal mice or mice given 1×10^5 B16 cells intravenously 2 wk previously, were infused into syngeneic C57BL/6 mice bearing B16 pulmonary metastases induced intravenously. Lungs were harvested 15 d after tumor induction and the metastases counted blindly. Control animals received no treatment.

surface phenotypes of the precursor and effector cells of the LAK cell mediating these in vivo effects were determined by depletion of Thy-1.2$^+$ cells before or after LAK cell generation. Fig. 6 demonstrates that treatment with complement (C′) plus anti–Thy-1.2 (θ) antibody before or after incubation in lymphokine abolished the generation of the cells responsible for the antimetastatic effects in vivo ($p < 0.05$). Transfer of LAK cells treated with C′ alone led to an equivalent decrease in metastases as transfer of LAK cells treated with neither antibody nor C′. Here again, the mice treated with cytoxan alone or cytoxan and either fresh (normal) splenocytes or splenocytes incubated in CM alone had the same number of metastases as the control group of mice without treatment (Fig. 6). The in vitro tumor lysis induced by lymphokine incubation was also abolished by treatment with anti–Thy-1.2 antibody plus C′ before or after incubation (3 ± 4% or 5 ± 1% vs. 72 ± 7% for the untreated LAK cells and 68 ± 4% or 75 ± 6% for the LAK cells treated with C′ alone before or after incubation). The fresh splenocytes and splenocytes in CM alone also had no significant in vitro cytotoxicity for tumor (7 ± 4% and 9 ± 5%, respectively). Thus, T cells are the effector and precursor cells for the in vivo antimetastatic and in vitro cytotoxic LAK cells.

LAK Activation in Various Growth Factors. The lymphokine required for generation of the cell effective in vivo was investigated using IL-2–containing supernatants from various sources for LAK activation. Fig. 7 shows that the

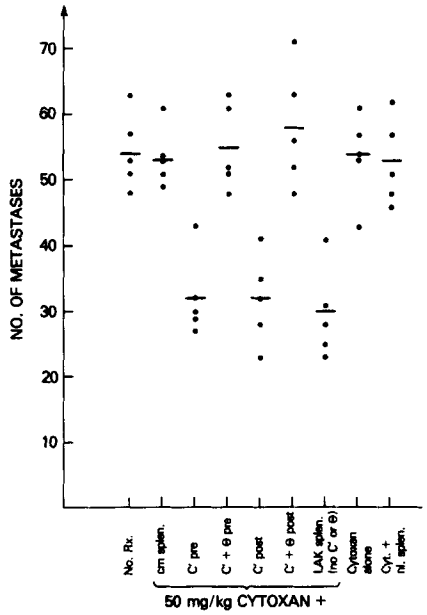

FIGURE 6. Phenotype of the precursors and effectors of the cells active in vivo. C57BL/6 mice were induced with 1×10^5 B16 cells intravenously. On day 3, they were infused with 50 mg/kg cytoxan and 6 h later, received either no treatment, 5×10^7 fresh (normal) splenocytes, 5×10^7 splenocytes incubated in CM alone, 5×10^7 untreated LAK cells (generated in Con A pulse Il-2), or 5×10^7 LAK cells treated with complement (C′) alone or with C′ plus anti–Thy-1.2 (θ) antibody either before or after lymphokine activation. The lungs were then assayed as described in the test. Control animals received no therapy.

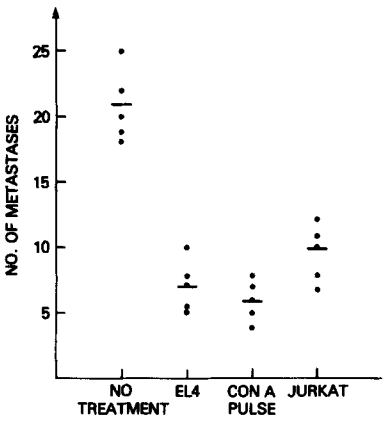

FIGURE 7. Activation in various IL-2–containing media. C57BL/6 mice, induced with 1×10^5 B16 cells intravenously, were infused on day 3 with 5×10^7 splenocytes activated in partially purified EL-4 or Con A pulse IL-2 or pure human Jurkat IL-2 with control animals receiving no treatment. Metastases were counted on day 15 as described in the texts.

human Jurkat IL-2 (purified to molecular homogeneity) led to equivalent generation of cells active in vivo as did the partially purified Con A pulse or EL-4 IL-2, all of which activated cells capable of significantly decreasing the number of B16 metastases ($p < 0.01$). Furthermore, incubation in all of the various IL-2 preparations led to the induction of equivalent levels of in vitro antitumor cytotoxicity (48 ± 4% for the Con A pulse IL-2, 54 ± 7% for the EL-4 IL-2, and 41 ± 6% for the pure Jurkat IL-2). Thus, the lymphokine responsible for the generation of the cells effective in vivo is probably IL-2.

Discussion

Numerous reports have suggested that lymphocytes specifically sensitized in vitro to primary or metastatic tumor cells are capable of antitumor effects in vivo (1–3, 19). However, such highly purified and immunogenic tumor cells are frequently unavailable in the human. Furthermore, several investigators have demonstrated antigenic heterogeneity between primary and metastatic tumors, with subsequent resistance to lysis by sensitized syngeneic lymphocytes (20, 21). Such problems led us to seek alternative methods of generating lytic cells with antitumor reactivity, e.g. incubation in lymphokines (4, 5), lectins (6, 7), or mixed lymphocyte cultures (8). We have now developed an in vivo tumor model in order to test whether this approach of activating cells in vitro without requiring tumor in the culture was capable of generating cells active in vivo. This paper represents the first report of the therapeutic efficacy in vivo of systemically administered LAK cells against established metastatic tumor.

The intravenous adoptive transfer of LAK cells, generated within 3 to 4 d in vitro by incubating splenocytes in IL-2, can lead to a significant decrease in the number of established B16 melanoma pulmonary metastases. This was found to be true whether the metastases were generated by intravenous injection of the B16 tumor cells or spontaneously from a primary tumor that was removed before LAK cell transfer. The latter model is of particular clinical relevance, since the

observation of pulmonary metastases after amputation of a primary tumor is often made. Important to the therapeutic relevance of these LAK cells is the finding that they were equally active in vivo even when they were generated from spleens of animals bearing large tumors. This equivalence of tumor-bearer and normal splenocytes through this activation is encouraging in the face of numerous reports suggesting the presence of suppressor cells in hosts with neoplasia, especially in its late stages (22–24).

The antitumor effects seen in vivo after administration of LAK cells leads to a significant increase in the survival time. Radiation of the mice was performed before tumor injection in the experiments determining survival advantage in an attempt to diminish any in vivo suppressor activity (18). In this regard it is of interest that the administration of cytoxan in vivo before LAK infusion did not increase the antitumor effects in spite of the large number of reports demonstrating the presence of cytoxan-sensitive cells that can enhance tumor growth and suppress in vitro or in vivo cell-mediated responses (25, 26). It appears that in this model, the cytoxan-sensitive suppressor cells do not play a major role.

It appears that NK cells do not play a major role in this therapeutic model. This is evidenced by the inability of the large number of fresh splenocytes transferred as controls to mediate any antitumor effects in vivo, unlike the data in some other tumor models (27, 28). Also, the phenotype of the precursor and effector cell active in vivo appears to be that of a T cell, and, in fact, the in vitro tumor lysis is also mediated by cells bearing the T cell phenotype (29, 30). Though we have determined that the cell mediating in vitro tumor lysis is Lyt-2^+, we have not determined the Lyt phenotype of the cells active in vivo.

In order to determine the mechanism of in vitro generation of active cells, we have demonstrated that the generation of the cells active in vivo is not due to any xenogeneic serum components or other tissue culture artifacts of the medium of incubation, a possibility that concerned us due to the reports of activation of immune cells by fetal calf serum or 2-mercaptoethanol alone (31, 32). In fact, it appears that some IL-2–containing medium is required for the generation of the therapeutic cells, and the efficacy in vivo of the splenocytes activated by the pure Jurkat IL-2 suggests that it is the IL-2 molecule itself that is responsible for this generation.

The mechanism of the antitumor effects in vivo of the LAK cells is unknown at present. It is possible that it is due to direct antitumor cytotoxicity, since the cytotoxic LAK cells are active in vivo, while the noncytotoxic fresh splenocytes or splenocytes incubated in CM are not. In most models of antitumor immunity using specifically sensitized splenocytes, the Ly-1^+ or helper class of lymphocytes appears to be instrumental (2), possibly by the recruitment of host effector cells through local production of lymphokines (33). It is possible that the activated LAK cells and not the control cells (fresh splenocytes or splenocytes incubated in CM alone) express such lymphokines in vivo.

Finally, the reason for our inability thus far to completely cure mice of the B16 pulmonary metastases is unknown. It is possible that the migration of such activated cells is not optimal, with little or no lymphatic recirculation or abundance at sites of tumor as evidenced by the abnormal homing patterns of T cell clones and lines (34, 35) or of IL-2–activated cells in the human (36). Perhaps

methods of local delivery of such cells, or overwhelming their normal in vivo clearance mechanisms may be clinically advantageous (37). It should be emphasized that we have only studied the ability of LAK cells to diminish lung metastases and cannot comment on the ability of these cells to affect metastases at other sites.

The presence of suppressor cells within the infused LAK population is another possible problem and may be overcome by the removal of tumor-enhancing cells (38). It is also likely that the activity of the LAK cells in vivo is short lived, similar to their rapidly vanishing cytotoxic activity in vitro when removed from IL-2 (data not shown). This would then necessitate the concomitant in vivo administration of IL-2, as shown in some other systems (39). Studies investigating these therapeutic protocols are in progress.

Summary

In previous in vitro studies, we have shown that murine splenocytes or cancer patient lymphocytes incubated in IL-2 become lytic for fresh syngeneic or autologous tumors. We have now performed the adoptive transfer of such lymphokine-activated killer (LAK) cells in a murine B16 metastasis model to test their in vivo efficacy. 1×10^8 LAK cells, infused intravenously into C57BL/6 mice with established B16 pulmonary metastases, led to a marked decrease in the number of lung nodules and improved survival. LAK cells administered 3 d after amputation of a tumor-bearing limb also decreased the incidence of spontaneous pulmonary metastases. LAK cells generated from tumor-bearer splenocytes had effects equivalent to those from normal animals, and this antimetastatic effect of the LAK cells did not require the prior administration of cyclophosphamide or other immunosuppressants. Fresh or unstimulated splenocytes had no effect. The antitumor effectors and precursors in vivo and in vitro were Thy-1^+. The lymphokine required for the activation appeared to be interleukin 2 (IL-2), since incubation in partially purified supernatants from PMA pulsed EL-4 or Con A–pulsed splenocytes or purified Jurkat IL-2 led to the generation of LAK cells equally active in vivo. The use of IL-2–activated cells may provide a valuable method for the adoptive therapy of human neoplasms as well.

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