Enhanced Antimetastatic Activity of Lymphokine-activated Killer Cells Purified and Expanded by Their Adherence to Plastic

Roderich E. Schwarz, Nikola L. Vujanovic, and John C. Hiserodt

Department of Pathology and Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

ABSTRACT

We have recently reported a simple and reproducible technique for the purification and rapid expansion of homogeneous populations of large granular lymphocytes expressing a natural killer cell phenotype and high levels of broad antitumor cytotoxic activity [lymphokine-activated killer (LAK) activity]. This technique exploits the observation that, in the presence of recombinant interleukin 2 (rIL-2), large granular lymphocytes/natural killer cells become adherent to plastic surfaces, actively proliferate, and acquire high levels of LAK activity. Because of their adherent properties these cells have been termed adherent LAK or A-LAK cells. The present studies investigate the antitumor effects of A-LAK cells in a syngeneic rat model of experimental pulmonary and hepatic metastases. For pulmonary metastases, F344 rats received i.v. injections with a natural killer-resistant mammary adenocarcinoma, MADB106, and, for hepatic metastases, animals received an intrasplenic injection of MADB106 tumor cells followed by surgical splenectomy. Three days later, the animals were treated with A-LAK cells alone, A-LAK cells plus rIL-2, or rIL-2 alone. These treatments were compared to immunotherapy using standard cultures of LAK cells (unfractionated spleen cells) and rIL-2. The results indicate that the administration of unfractionated LAK cells plus interleukin 2 (IL-2) was effective in reducing established lung or liver metastases in this rat model. However, the results also indicate that purified populations of A-LAK cells in combination with rIL-2 demonstrate dramatic and superior antitumor effects when compared to LAK cells cultured under standard conditions. The antitumor effects of standard LAK cells or A-LAK cells plus IL-2 translated into significant survival benefits compared to animals receiving no therapy or IL-2 therapy alone. Survival after therapy with A-LAK cells plus IL-2 was significantly prolonged compared to treatment with standard LAK cells. These data suggest that purified populations of LAK cells (derived from natural killer cells) may prove superior for adoptive immunotherapy in the clinical setting.

INTRODUCTION

Adoptive Immunotherapy

Effective AIT using LAK cells plus rIL-2 has recently gained much attention due to the observed antitumor activity in experimental animal models (1-4) and in some cancer patients (5-8). LAK cells are generated by the culture of lymphocytes derived from normal or cancer patients in crude IL-2 or rIL-2 (9, 10). Effective AIT using LAK cells plus rIL-2 requires the administration of large numbers of cultured lymphocytes and high (often toxic) levels of rIL-2. These limitations have compromised the general use of this form of immunotherapy in the clinical setting. Moreover, because the actual frequency of the precursors of LAK effector cells within a population of peripheral blood lymphocytes is generally around 10% (11-14), it has not been possible to purify these cells to obtain sufficient numbers to be used for therapy. A major hypothesis has suggested, however, that if unfractionated populations of IL-2-activated lymphoid cells could mediate beneficial antitumor effects, then purified LAK effector cells should mediate even better effects. In addition, the use of purified populations of LAK cells would allow investigations into additional areas of study, such as in vivo migration patterns, cytokine production, and cellular interactions responsible for the LAK effect. Therefore, the development of simple and reproducible techniques in which the LAK effector cells could be purified and expanded to sufficient numbers to be used for therapy yet require a lower level of rIL-2 to support their antitumor activity would provide an alternative and presumably preferable technique to current culture methods.

We have recently reported a simple technique for the isolation and rapid expansion of purified populations of LGL/NK cells with high levels of broad antitumor (LAK) cytotoxic activity (15). This technique involves the adherence of LGL to plastic surfaces induced by rIL-2. A-LAK cells expand rapidly in culture (up to 100-fold in 4 to 5 days) and generate exceedingly high levels of LAK cytolytic activity.

The present study analyzes the antitumor activity of A-LAK cells against 3-day established lung and hepatic metastases of an NK-resistant mammary adenocarcinoma, MADB106. The results demonstrate that A-LAK cells are superior to standard cultures of LAK cells in mediating the reduction of experimental metastases as well as in conferring increased animal survival.

MATERIALS AND METHODS

Animals

Male Fischer 344 rats (75-100 g) were purchased from Taconic Farms (Germantown, NY) and were housed in a specific-pathogen-free animal facility at the Pittsburgh Cancer Institute.

Culture Medium and Tumor Cells

Standard culture medium consisted of RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% FCS, antibiotics (streptomycin/penicillin), and L-glutamine. Medium for growing LAK cells was standard medium additionally supplemented with 5 x 10^{-2} m 2-mercaptoethanol and 500 units/ml of rIL-2 (LAK medium). Tumor cells consisted of the NK-resistant mammary adenocarcinoma MADB106 (16) and an LGL leukemia, CRNK-16 (17), both syngeneic in F344 rats. The murine mastocytoma, P815, was also used as an indicator of LAK activity, and the Moloney virus-induced YAC-1 T cell lymphoma was used as the indicator of NK activity. All tumor cells were grown in standard medium and subcultured 3 times a wk.

Preparation of Lymphoid Cells

Spleens were aseptically removed, and single cell suspensions were prepared in RPMI 1640 with 10% FCS. Splenic mononuclear cells...
were obtained after centrifugation on Ficoll-Hypaque gradients (density = 1.077) at 300 x g for 20 min. Except for the use of mononuclear cells in unfractioned bulk cultures, the mononuclear leukocytes were passed over nylon wool columns to remove monocytes/macrophages and B-cells (18). Thus, 10³ spleen cells in 2 ml of RPMI 1640:10% FCS were added to a 30-ml syringe containing 0.6 g of sterile nylon wool (Cellular Products, Buffalo, NY). The cells were incubated for 1 h at 37°C, and the nylon wool was gently washed (without squeezing) with 30 ml of 37°C RPMI 1640:10% FCS. The nonadherent cells were collected, washed, and used. By this procedure, we consistently reduced the percentage of B-cells in the spleen preparations to less than 2% (by flow cytometric analysis using anti-immunoglobulin antibodies) and the percentage of monocyte/macrophages to less than 0.3% (by morphological analysis of Giemsa-stained cytocentrifuge preparations).

Generation of Cells with LAK Activity

Standard Cultures. Cells with LAK activity were produced by culturing unfractionated splenic lymphoid cells at 2 x 10⁶ cells/ml in LAK medium for 5 days at 37°C.

Adherence Cultures. The details of the methods for generating and expanding adherent LAK cells from F344 rats have been reported (15). Briefly, 200 x 10³ nylon wool-nonadherent mononuclear leukocytes were cultured in 100 ml of LAK medium (containing 500 units/ml of rIL-2) in 150-cm² flasks (GIBCO). The cells were cultured for 48 h at 37°C after which the nonadherent cells were decanted and the adherent cells were washed 3 times with 20 ml of warm (37°C) RPMI 1640 containing 2% FCS. The adherent cells then received the conditioned medium from which they were initially cultured. This conditioned medium was prepared by removing the nonadherent cells by centrifuging and passing the medium through a 0.45-µm Millipore filter. The cultures were then incubated at 37°C for an additional 3 days. To remove the adherent LAK cells, the medium was decanted and 5 ml of ice-cold 5 mM EDTA in phosphate-buffered saline were added for 2 min; the cells were collected, washed, counted, and used.

Antibodies Used

A panel of antibodies was used in these studies. These included the mouse IgG₁ monoclonal antibodies OX19 (CD5), OX8 (CD8), W3/25 (CD4), OX6 (Ia), and OX39 (CD25, IL-2 receptor), all purchased from Accurate Scientific (Westbury, NY). Each of these antibodies was used at a 1:100 dilution based on preliminary dose-response titrations. Anti-asialo-GM₁ was purchased from Wako Chemical Company (Dallas, TX) and used at a 1:200 dilution. Affinity-purified IgG of rabbit anti-laminin antiserum (anti-p48) was produced in our laboratory, and its reactivity with NK cells and A-LAK cells has been previously described (14, 19, 20). A mouse IgG₁(k) monoclonal antibody termed 3.2.3), which reacts with all rat LGL/NK cells and A-LAK cells but not T-cells, was produced in our laboratory. All second step reagents were fluorescein isothiocyanate-labeled F(ab')² fragments of goat antibody against the primary antibody and were purchased from Cappel (Malvern, PA).

Flow Cytometry

For surface marker analysis, 2 x 10⁶ lymphocytes were placed in 12- x 75-mm glass tubes in 0.1 ml of staining buffer [phosphate-buffered saline (pH 7.3):0.1% sodium azide:2% FCS]. Various antibodies were added to the cells for 30 min at 4°C. The cells were washed twice, incubated with fluorescein isothiocyanate-labeled second antibodies, washed, and resuspended on 1% paraformaldehyde prior to analysis for fluorescence on a FACStar flow cytometer (Becton-Dickinson, Cambridge, MA). The target cells were labeled with 100 µCi of Na₂⁵¹CrO₄ per 2 x 10⁶ cells, washed, and seeded into 96-well culture dishes at 5 x 10³ cells/well. Suspensions of effector cells were then added to triplicate wells to give various effector:target ratios in a final volume of 200 µl. After an additional incubation at 37°C for 4 h, 100 µl of supernatant were removed from each well and counted in a gamma counter to determine experimental release (ER). Spontaneous release (SR) was obtained from wells receiving target cells and medium only, and total release (TR) was obtained from wells receiving 1% Triton X-100. The percentage of cytotoxicity was calculated by the following formula:

\[
\% \text{ of cytotoxicity} = \frac{(ER - SR)}{(TR - SR)} \times 100
\]

Lytic units of cytotoxic activity were determined from linear regression curves plotted from various effector:target ratios. In all cases, 1 lytic unit was defined as the number of effector cells required to cause 20% specific ⁵¹Cr release from 5 x 10⁶ target cells. Total lytic units per culture were calculated by adjusting the lytic units/10⁶ cells value to the total number of cells in the culture.

Statistics

Statistical analysis of the differences between treatment groups was performed using the nonparametric Kruskal-Wallis test. Pairwise comparisons between individual groups were done with Mann-Whitney rank sum tests. P < 0.05 was considered significant.

Adoptive Immunotherapy

Pulmonary Metastases. F344 rats received i.v. injections in the lateral tail vein with 7 x 10³ viable MADB106 tumor cells in 1 ml of Ca²⁺- and Mg²⁺-free HBSS. After 3 days, the animals received a second i.v. (tail vein) injection of graded numbers of LAK effector cells also suspended in 1 ml of HBSS. The animals then received i.p. injections of rIL-2 (2 ml of HBSS containing 20 to 50 x 10⁶ units of rIL-2) twice daily for 5 days. After 18 to 21 days the animals were sacrificed by CO₂ asphyxiation, the lungs were inflated with India ink and bleached in Fekete's solution, and the pulmonary metastases were counted and photographed. For survival studies, all animals were autopsied to determine the extent of metastatic disease.

Hepatic Metastases. F344 rats were anesthetized with fluorothane gas, the spleens were surgically exposed, and 4 x 10⁶ MADB106 tumor cells in 2 ml of Ca²⁺- and Mg²⁺-free HBSS were injected intraspinally. The spleen was then surgically removed after 5 min. Three days later, the animals received AIT identical to that described above. Hepatic metastases were determined 18 to 21 days later after intracardiac injection of India ink, removal of the livers, and bleaching in Fekete's solution.

RESULTS

Phenotype of the A-LAK Cells. Fig. 1 shows the surface marker phenotype of A-LAK cells generated from F344 spleen cells with respect to 7 markers commonly used in the analysis of rat lymphocyte subsets. It is obvious that 90 to 100% of the purified A-LAK cells expressed OX8, asialo-GM₁, laminin (p48), and the structure identified by monoclonal antibody 3.2.3 (Fig. 1, E to H), surface markers which are all common to rat LGL/NK cells. In contrast, neither OX19 (CD5), W3/25 (CD4), nor OX41 (macroparticle specific) was expressed on these cells, consistent with previous reports regarding the phenotype of LAK effector cells in rats (14). In data not shown, A-LAK cells also failed to express detectable levels of OX39 (the CD25 equivalent in rats) or surface immunoglobulin but did express the OX6 (Ia) surface marker. Finally, all A-LAK cells were LGL. In contrast to these results, cultures of standard LAK cells (unfractionated spleen cells) contained mainly OX19⁺ cells (up to 40 to 60%) and immunoglobulin⁺ cells (30 to 50%), with

ENHANCED ANTIMETASTATIC ACTIVITY OF ADHERENT LAK CELLS

only around 10 to 20% 3.2.3+ LGL (data not shown).

In Vitro Cytolytic Activity of A-LAK Cells. Table 1 shows the in vitro cytolytic activity of purified A-LAK cells against a panel of tumor target cells as well as normal PBL target cells. Cytolytic activity of A-LAK cells was compared to that mediated by standard LAK cells generated in bulk cultures. It is clear that A-LAK cells demonstrate very high levels of in vitro cytotoxic activity against all tumor targets tested (including syngeneic NK-resistant MADB106 and CRNK-16) while lower levels of activity against all tumor target cells tested. Additional studies using cell sorting have shown that the cytolytic activity within the standard LAK cultures is, in fact, mediated by cells which are phenotypically identical to the purified A-LAK cells (14). Thus, A-LAK cells represent purified effector cells displaying similar per cell lytic activity compared to effector cells present in standard unfractionated LAK cultures.

Antimetastatic Effects of A-LAK Cells. Using models of established 3-day pulmonary or hepatic metastases of an NK-resistant mammary carcinoma, MADB106, we analyzed the antimetastatic activity of A-LAK cells and compared their activity to LAK cells generated under standard conditions. The results of these experiments are shown in Fig. 2. In general, the i.v. administration of 10^7 purified A-LAK cells mediated up to 90% reduction of pulmonary metastases, with some animals showing as few as 2 lung metastases. This result was in contrast to the antimetastatic effects seen with LAK cells generated under standard conditions in which a single i.v. injection of 50 x 10^6 cells, representing similar in vitro lytic activity against MADB106 tumor cells, demonstrated a significant (50% reduction), albeit reduced, effect. The administration of rIL-2 alone at the level given to support the A-LAK effect (0.5 x 10^6 units/kg, i.p., twice a day) showed slight but not significant (P > 0.08) antimetastatic effects in this pulmonary model. In some experiments, such as the one shown in Table 2, the administration of IL-2 alone showed a weakly significant antimetastatic effect but only at the highest level given (Mann-Whitney test, P = 0.035 at 10^6 units/kg/day). When the A-LAK cells were titrated, we found that as few as 3 x 10^6 cells could mediate significant antimetastatic activity against Day 3 MADB106 pulmonary metastases (Table 3). Interestingly, higher levels of A-LAK cells (2 x 10^7 and above) yielded reduced antitumor efficacy. Results similar to those obtained with pulmonary metastases were noted when A-LAK cells were used in the therapy of 3-day established hepatic metastases (Fig. 2B), except that with hepatic metastases the administration of IL-2 alone gave a slightly significant antimetastatic effect (P > 0.02).

Additional experiments were performed to determine whether the observed antimetastatic effects of A-LAK cells would translate into significant extension of animal survival after tumor injections. The results shown in Fig. 3 indicate that a significant survival benefit is observed in treatment groups receiving either unfractionated LAK cells or A-LAK cells together with IL-2, although superior survival was noted in those animals receiving the A-LAK cells (P = 0.0019 for lung metastases and P = 0.0306 for liver metastases).

IL-2 Requirement for Optimal Antimetastatic Activity of Purified A-LAK Cells. In order to determine the optimal levels of rIL-2 required to mediate optimal antimetastatic activity in this model, a constant number of A-LAK cells were administered (10^7 cells), and varying doses of rIL-2 were given. The results of these experiments are shown in Table 2. When 10^7 A-LAK cells were administered together with varying doses of rIL-2, we found that optimal antimetastatic activity could be supported with as low as 0.1 x 10^6 total units/kg/day. Elimination of the rIL-2 altogether (A-LAK cells alone) or elimination of the A-LAK cells (IL-2 alone) only resulted in weakly significant antimetastatic effects (see statistical analysis in Table 2).

Table 1 In vitro cytotoxic activity of A-LAK cells and standard LAK cells (unfractionated spleen cells) against a panel of tumor and normal target cells

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>YAC-1</th>
<th>P815</th>
<th>MADB106</th>
<th>CRNK-16</th>
<th>PBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-LAK</td>
<td>3900 ± 260d</td>
<td>1665 ± 190</td>
<td>1896 ± 140</td>
<td>420 ± 25</td>
<td>&lt;10 ± 5</td>
</tr>
<tr>
<td>Standard LAK</td>
<td>890 ± 50</td>
<td>360 ± 20</td>
<td>497 ± 55</td>
<td>90 ± 15</td>
<td>&lt;10 ± 5</td>
</tr>
</tbody>
</table>

- Effector cells were generated from F344 spleen cells as described in "Materials and Methods."
- LU50, lytic unit; PBL, peripheral blood lymphocytes.
- Four-h 51Cr release assay using effector:target ratios of 30:1, 10:1, and 3:1.
- dMean ± SD.

Fig. 1. Flow cytometric analysis of surface markers expressed on purified A-LAK cells. OX19 = CD5; W3/25 = CD4; OX41 = macrophage specific; OX8 = CD8; asialo-GM1 = LGL/NK cells, some T-cells, and macrophages; p48 = LGL/NK cells; and 3.2.3 = LGL/NK cells, A-LAK cells, and neutrophils.

Fig. 2. Antimetastatic effects of A-LAK cells combined with IL-2. Table 2. When 10^7 A-LAK cells were administered together with varying doses of rIL-2, we found that optimal antimetastatic activity could be supported with as low as 0.1 x 10^6 total units/kg/day. Elimination of the rIL-2 altogether (A-LAK cells alone) or elimination of the A-LAK cells (IL-2 alone) only resulted in weakly significant antimetastatic effects (see statistical analysis in Table 2).
Fig. 2. Adoptive immunotherapy of established MADB106 pulmonary or hepatic metastases using A-LAK cells. F344 rats (eight animals/group for lung metastases and five animals/group for liver metastases) received MADB106 tumor cells as described in "Materials and Methods," and 3 days later received either no treatment (Group C); rIL-2 alone (0.5 x 10^6 units/kg/day) (Group I); 50 x 10^6 standard LAK cells plus rIL-2 (Group S); or 10^7 A-LAK cells plus rIL-2 (Group A). Lung metastases were counted on Day 18, liver metastases on Day 21. Data from all animals are shown.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Groups compared</th>
<th>Kruskal-Wallis</th>
<th>Mann-Whitney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>C-I-S</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-I-A</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I-S</td>
<td></td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td>I-A</td>
<td></td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td>S-A</td>
<td></td>
<td>0.0009</td>
</tr>
<tr>
<td>Liver</td>
<td>C-I-S</td>
<td>0.0049</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-I-A</td>
<td>0.0025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I-S</td>
<td></td>
<td>&gt;0.06</td>
</tr>
<tr>
<td></td>
<td>I-A</td>
<td></td>
<td>0.0122</td>
</tr>
<tr>
<td></td>
<td>S-A</td>
<td></td>
<td>0.0367</td>
</tr>
</tbody>
</table>

DISCUSSION

LAK cells, generated by the culture of lymphoid cells in crude or rIL-2, have the capacity to lyse a variety of tumor target cells including fresh or cultured autologous solid tumor targets, leukemic blasts, or allogeneic or xenogeneic tumor cells while sparing, for the most part, normal cells. When combined with the administration of high levels of IL-2, LAK cells can mediate the reduction of established tumor metastases in animal models and in some cancer patients. Although AIT using LAK cells plus IL-2 has gained much attention due to these effects, the responses are often transient and require very large numbers of effector cell preparations. Moreover, because of the high levels of IL-2 needed to support the large numbers of transferred lymphocytes, severe and often life-threatening toxicities can be encountered (6, 21, 22). Therefore, developing strategies of administration of purified populations of LAK effector cells together with lower levels of IL-2 would provide an alternative and presumably better approach to the standard form of LAK therapy.

This study represents the first to analyze the therapeutic efficacy of LAK cells in a rat model of established tumor metastases. Our results are similar to those previously reported using standard mouse models with LAK treatment of Day 3 micrometastases (1, 2, 8). Moreover, the antimetastatic efficacy of standard LAK cells was compared to that of a highly enriched population of LAK cells we have termed A-LAK cells. The results unequivocally demonstrate that AIT using purified populations of LAK effector cells yields enhanced antimetastatic effects compared to standard LAK cells. Moreover, these observed antimitastatic effects are translated into increased animal survival. The population of LAK effector cells used in these studies was purified from F344 rat spleen cells by a simple and reproducible technique recently developed in our laboratory (15). This technique exploits the observation that, when LGL/NK cells are stimulated with IL-2, they become adherent to plastic surfaces. Moreover, when plastic-adherent LGL/NK cells are grown in IL-2-containing conditioned medium, they proliferate briskly (up to 100-fold in 4 to 5 days) to generate
Table 2 Effects of different doses of administered rIL-2 on the in vivo antimitastatic activity of A-LAK cells

F344 rats (six rats/group) received 7 x 10^6 MABDB106 tumor cells i.v. on Day 0. Three days later the rats received a single i.v. injection of 10^7 A-LAK cells, followed by 5 consecutive days of rIL-2 given i.p., twice daily. Lung metastases were counted on Day 21.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total administered rIL-2/kg/day (units)</th>
<th>Lung metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td></td>
<td>225 ± 32^a</td>
</tr>
<tr>
<td>B</td>
<td>rIL-2 alone</td>
<td>10^6</td>
<td>178 ± 20</td>
</tr>
<tr>
<td>C</td>
<td>rIL-2 alone</td>
<td>0.5 x 10^6</td>
<td>202 ± 26</td>
</tr>
<tr>
<td>D</td>
<td>rIL-2 alone</td>
<td>0.1 x 10^6</td>
<td>216 ± 32</td>
</tr>
<tr>
<td>E</td>
<td>A-LAK + rIL-2</td>
<td>10^6</td>
<td>6 ± 5</td>
</tr>
<tr>
<td>F</td>
<td>A-LAK + rIL-2</td>
<td>0.5 x 10^6</td>
<td>5 ± 5</td>
</tr>
<tr>
<td>G</td>
<td>A-LAK + rIL-2</td>
<td>0.1 x 10^6</td>
<td>48 ± 23</td>
</tr>
<tr>
<td>H</td>
<td>A-LAK alone</td>
<td></td>
<td>164 ± 87</td>
</tr>
</tbody>
</table>

Table 3 Dose-response effects of treating 3-day established MABDB106 lung tumors with different numbers of purified A-LAK cells plus rIL-2

F344 rats (six rats/group) received 7 x 10^6 MABDB106 tumor cells on Day 0. Three days later they received a single i.v. injection of A-LAK cells and 5 consecutive days of rIL-2 (0.5 x 10^5 units/kg, i.p., twice daily). Lung metastases were counted on Day 20.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of A-LAK cells given</th>
<th>No. of lung metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None</td>
<td>112 ± 25^a</td>
</tr>
<tr>
<td>B</td>
<td>1 x 10^7</td>
<td>13 ± 8</td>
</tr>
<tr>
<td>C</td>
<td>2 x 10^7</td>
<td>9 ± 7</td>
</tr>
<tr>
<td>D</td>
<td>2 x 10^7</td>
<td>28 ± 11</td>
</tr>
</tbody>
</table>

Fig. 3. Kaplan-Meier survival curves after adoptive immunotherapy of Day 3 pulmonary (A) and hepatic (B) micrometastases. Experimental protocol was similar to that described in Fig. 2. O, no treatment; •¿, IL-2 alone (0.5 x 10^5 units/kg/day); A, 50 x 10^7 standard LAK cells plus IL-2; A, 10 x 10^7 A-LAK cells plus IL-2.

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Kruskal-Wallis</th>
<th>Mann-Whitney</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B-C-D</td>
<td>0.0354</td>
<td></td>
</tr>
<tr>
<td>B-C-D</td>
<td>&gt;0.055</td>
<td></td>
</tr>
<tr>
<td>A-E-F-G-H</td>
<td>0.00001</td>
<td></td>
</tr>
<tr>
<td>E-F-G-H</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>E-F</td>
<td>&gt;0.8727</td>
<td></td>
</tr>
<tr>
<td>E-G</td>
<td>0.0104</td>
<td></td>
</tr>
<tr>
<td>F-G</td>
<td>0.0082</td>
<td></td>
</tr>
<tr>
<td>A-H</td>
<td>0.0453</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD.
This possibility is supported by recent findings demonstrating that the coculture of human or rat tumor cells with moderate to low levels of γ-interferon made the cells resistant to LAK cytotoxic effects (23–25). Certainly A-LAK cells produce very high levels of γ-interferon.6

These studies in rats may also have important implications for AIT in humans. The adherence technique has been applied to mice as well as humans (26) with the resulting development for AIT in humans. The adherence technique has been applied to large numbers of purified A-LAK cells. Thus, the technique is a general one, applicable to all species under study. In mice, we have found that A-LAK cells also contain potent antimetastatic activity, corroborating the results reported here using a rat model.7 We are currently investigating the antimetastatic activity of human A-LAK cells in a Phase I clinical trial.

REFERENCES


Enhanced Antimetastatic Activity of Lymphokine-activated Killer Cells Purified and Expanded by Their Adherence to Plastic

Roderich E. Schwarz, Nikola L. Vujanovic and John C. Hiserodt


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/49/6/1441

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.