Regulation of the Metastasis of Murine Ocular Melanoma by Natural Killer Cells

Ronald Horning,* Gloria C. Koo,t and Jeanne Szalay*

In the current study we examine parameters affecting the metastasis of ocular tumors of in vivo derived B16F10 melanoma. In C57BL/6J beige (bg/bg) mice, with low NK activity, metastasis to the lungs was increased and survival time decreased. In C57BL/6J normal (+/+) mice treatment with PK136, a highly specific monoclonal anti-NK antibody (Ab), caused a depletion of NK cytotoxic activity, as demonstrated using a standard 51Cr release assay. In animals bearing ocular tumors, treatment with PK136 Ab resulted in significantly increased pulmonary metastasis and an altered pattern of metastasis. The effect of combined treatment protocols using LS2616 (Linomide) and cyclophosphamide (Cy) was examined in enucleated and unenucleated animals. Treatment with LS2616 and Cy resulted in a significant decrease in mean pulmonary metastases (MPM), a decreased frequency of metastasis to the submandibular lymph nodes and an increase in mean survival time. In enucleated mice this combined treatment protocol resulted in apparent cures, the lowest MPM and the longest survival time observed. When tumor-bearing mice were treated with either silica, carrageenan or sublethal gamma irradiation, no effect on metastasis or survival was observed. This study demonstrates the importance of the NK cell as a primary effector cell for the control of metastasis from in vivo derived ocular B16F10 melanoma. Invest Ophthalmol Vis Sci 30:1909-1915, 1989

The role of cellular immunity in the regulation of metastasis from ocular murine melanoma has been examined by others using cultured B16F10 cells. In these experiments, the importance of cytotoxic T cells in regulating metastatic growth of in vitro derived ocular B16F10 melanoma in immune-compromised mice was demonstrated. NK cells did not appear to play a role in the regulation of metastasis in this system.1,2 In contrast, Yokoyama et al3 have shown that enhancement of NK activity with alpha-interferon resulted in significantly fewer pulmonary metastases from in vitro derived B16 melanoma in normal C57BL/6 mice. Therefore, it is still not clear if NK cells regulate metastasis in these systems.

LS2616, a newly discovered quinoline 3-carboxamide, has been shown by others to increase NK cytotoxic activity4 and reduce pulmonary metastases arising from subcutaneous (sc) and intravenous (iv) inoculations of in vitro derived B16F10 melanoma cells.5 More recently, we demonstrated the effectiveness of LS2616 (Linomide) in inhibiting metastasis of ocular and subcutaneous tumors of highly aggressive in vivo derived B16F10 tumor cells.6 In vivo derived cells differ from cultured B16F10 cells in spontaneously metastasizing from the eye of immune-competent C57BL/6J hosts.7 When combined with enucleation 7 days after intracameral (ic) inoculation of tumor cells, LS2616 therapy resulted in an apparent cure in some animals. In these experiments we noted that LS2616-enhanced splenic effector cells were capable of causing a significant reduction of pulmonary metastasis from ic and sc inoculations of in vivo derived B16F10 melanoma. The identity of the effector cell was not determined.

Monoclonal Ab PK136 detects NK1.1 antigen on NK cells and has been found to be useful in eliminating greater than 95% of splenic NK activity in C57BL/6J mice.8,9 PK136 Ab treatment does not alter other lymphocyte populations in the spleen, and does not affect T cell cytotoxicity or humoral immunity.10 This Ab has been used to demonstrate the role...
of NK cells in the regulation of experimental metastasis and survival in mice inoculated iv with in vitro derived B16F10 melanoma cells.\textsuperscript{10}

In the current experiments we examine parameters affecting metastasis of in vivo derived B16F10 ocular tumors and further explore the role of NK cells in the system.

Materials and Methods

Experimental Animals

Five- to 7-week-old C57BL/6J normal (+/+) and beige (bg/bg) mice (Jackson Laboratories, Bar Harbor, ME) of both sexes were used as experimental subjects. Normal C57BL/6J mice were used to maintain the tumor line in vivo. Tumor-bearing animals were examined as described earlier.\textsuperscript{6} Mice were maintained according to the recommendations outlined in the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council. These investigations conform to the ARVO Resolution on the Use of Animals in Research.

Tumor Maintenance and Preparation

The B16F10 melanoma cell line was obtained in 1984 from Dr. Artemio Ovejera (NIH, Bethesda, MD). Tumor cells were maintained in vivo as described previously.\textsuperscript{6} Tumor cell suspensions were prepared by gentle trypsinization (0.25% trypsin in DMEM, at 37°C in 5.0% CO\textsubscript{2} for 3 min) of 1 cm\textsuperscript{3} pieces of primary tumor grown in the flank. Cell suspensions were washed 2X in DMEM.

Establishment and Resection of Primary Tumors

Tumor inoculations and enucleations were carried out as described previously\textsuperscript{7} except that lower concentrations of tumor cells were used in the current experiments. Briefly, mice were anesthetized by intraperitoneal (ip) injection with Nembutal (Abbot Laboratories, North Chicago, IL) and a 5 \textmu l cell suspension of 10\textsuperscript{4} B16F10 tumor cells in DMEM was inoculated into the anterior chamber. Anesthetized mice were enucleated 10 days later (E10) and a suture used to close the eyelids. Enucleated eyes were always filled with tumor and recurrent growth of tumor in the orbit did not occur.

Drug Treatment and Irradiation

LS2616 was graciously provided by Dr. T. Stalhandske (AB Leo, Helsingborg, Sweden) and was administered to mice in drinking water at a dosage of 160 mg/kg/day as described previously.\textsuperscript{6} LS2616 treatment began 7 days prior to inoculation of tumor cells and continued for the duration of the experiment. Cyclophosphamide (Cy; Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline and administered ip weekly at a dose of 25 mg/kg. Treatment began 1 day after inoculation of tumor cells. Ip injections of either silica or carrageenan (Sigma Chemical Co.) at a dosage of 1 mg/mouse were administered weekly.\textsuperscript{3} Treatment began 1 day after inoculation of tumor cells. Mice were irradiated (500 rads, \textsuperscript{137}Cs) at a dosage of 117 rads/min.

Antibody Treatment

The derivation of monoclonal Ab PK136 has been described previously.\textsuperscript{8} Administration of PK136 Ab can be maintained for 20–24 weeks.\textsuperscript{9} A mouse IgG2a monoclonal Ab, H16-L10-4, was used in some experiments as a specificity control. This Ab recognizes influenza A virus nucleoprotein and is not known to react with mouse antigens.\textsuperscript{11} PK136 and H16-L10-4 were diluted 1:10 in sterile saline and 0.5 ml of diluted antibody was injected ip into mice. Mice were injected with Ab every 7–10 days, beginning 1 week before inoculation of tumor cells.

Assays

\textit{In vitro NK cytotoxicity:} Natural killer cell activity in spleen cells was assessed against YAC-1 tumor cells using a 4 hr \textsuperscript{5}Cr-release assay.\textsuperscript{9}

\textit{Metastasis:} In most experiments, mice either died or became moribund and were necropsied. However, in some protocols noted below, healthy animals were sacrificed and necropsied. In all experiments, the lungs, spleen, liver, intestine, lymph nodes, brain, heart, kidneys and adrenals were removed and examined at \times 25 for evidence of metastasis. The number of metastases was determined as described previously.\textsuperscript{12}

\textit{Mean survival time:} Mean survival time was calculated as the average number of days between tumor cell inoculation and either morbidity or the sacrifice and necropsy of apparently healthy mice at the end of 11 weeks. Frequency of survival was the number of mice alive and apparently healthy at the end of 11 weeks/number of mice in the group.

Statistical Analysis

Differences in the mean number of nodules or mean survival time were analyzed using Duncan’s Multiple Range Test (S.A.S. Institute, NC).
Results

Survival of bg/bg and Control (+/+) Mice Bearing Intracameral Tumors

The bg/bg mutation in the C57BL/6J mouse is an autosomal recessive mutation that results in severely depressed NK cell activity, decreased T cell function and decreased macrophage cytotoxicity. In the current experiments, bg/bg mice received ic inoculations of B16F10 tumor. Tumorigenicity and survival of hosts was compared to that observed in control (+/+) mice. In examining the effect of varying concentrations of tumor cell inocula on growth and metastasis in C57BL/6J (+/+) mice, we found that 10^2 tumor cells inoculated ic results in 100% tumorigenicity and pulmonary metastasis, and death within 5–6 weeks. We now routinely inoculate 10^2 tumor cells in most of our ic experiments. When nine bg/bg mice received ic inoculations of 10^2 melanoma cells, mean survival was 16.6 ± 1.8 days. In contrast, nine control (+/+) mice that received 10^2 tumor cells had a survival of 31.1 ± 1.6 days. When eight bg/bg mice received 10^3 tumor cells, the survival time was 22.3 ± 2.2 days. Control (+/+) animals receiving 10^3 cells ic survived 32.3 ± 1.7 days. At both tumor cell concentrations, survival was significantly decreased in bg/bg mice (Table 1). The mean survival of bg/bg mice inoculated ic with 10^2 tumor cells was not statistically different from that of bg/bg mice receiving 10^3 tumor cells. Tumorigenicity was 100% in all experimental and control groups.

Metastasis of Intracameral Tumors in bg/bg and Control (+/+) Mice

To determine the effect of the beige mutation on pulmonary metastasis in mice harboring ic tumors derived from in vivo derived B16F10 melanoma, bg/bg mice were inoculated ic with tumor cells and sacrificed when moribund. Control (+/+) mice received ic tumor cells and were sacrificed at the time that experimental animals had become moribund. Nine bg/bg mice received ic inoculations of 10^2 tumor cells and survived an average of 16.6 ± 1.8 days. Seven of nine had pulmonary metastases and the mean number of pulmonary metastases (MPM) was 30.5 ± 11.3. Six control (+/+) mice that received 10^2 tumor cells were sacrificed on day 17 and the MPM was 1.3 ± 0.7 (P < 0.01) (Table 2).

Eight bg/bg mice received ic inoculations of 10^3 melanoma cells and their survival was 22.3 ± 2.2 days. All eight animals had pulmonary metastases and the MPM was 120.0 ± 23.2. In contrast, only three of six control mice receiving 10^3 tumor cells ic survived an average of 16.6 ± 1.8 days. All eight animals had pulmonary metastases that experimental animals had become moribund. Control (+/+) mice receiving 10^3 tumor cells and were sacrificed at the time

Table 1. Survival of bg/bg and control mice inoculated intracamerally with in vivo derived B16F10 melanoma cells

<table>
<thead>
<tr>
<th>Genotype/ inoculum</th>
<th>Frequency</th>
<th>Mean days (±SEM)</th>
<th>Tumorigenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/IC 10^2</td>
<td>0/9</td>
<td>31.1 (±1.6)</td>
<td>9/9</td>
</tr>
<tr>
<td>Beige/IC 10^2</td>
<td>0/9</td>
<td>16.6 (±1.8)</td>
<td>9/9</td>
</tr>
<tr>
<td>Control/IC 10^3</td>
<td>0/8</td>
<td>32.3 (±1.7)</td>
<td>8/8</td>
</tr>
<tr>
<td>Beige/IC 10^3</td>
<td>0/8</td>
<td>22.3 (±2.2)</td>
<td>8/8</td>
</tr>
</tbody>
</table>

* Inoculum = control (+/+) or beige (bg/bg) mice received intracameral inoculations of either 10^2 or 10^3 B16F10 melanoma cells.
† Mean days = mean survival time as described in Materials and Methods.
‡ Tumorigenicity = number of mice that developed ocular tumors/number of mice per group.
§ P < 0.01.

and sacrificed at day 23 had pulmonary metastases and the MPM was only 2.4 ± 2.5 (P < 0.01).

The Effect of LS2616 and Cyclophosphamide on Survival and Metastasis in Enucleated or Unenucleated Tumor-Bearing Mice

The current experiments were designed to examine the effect of LS2616 and Cy on survival and tumor metastasis in mice harboring ic tumors. Treatment of unenucleated mice with LS2616 alone decreased both MPM and the incidence of SMLN, but had no effect on survival (Table 3). Cy alone had no effect on survival or metastasis. Combined treatment with LS2616 and Cy decreased the MPM and frequency of SMLN metastasis and increased survival. The decrease in pulmonary metastasis was significantly greater than that seen using LS2616 alone.

The effect of drugs was also examined in animals whose tumor-filled eyes were enucleated 10 days postinoculation with tumor cells. Enucleation alone

Table 2. Pulmonary metastasis of in vivo derived B16F10 melanoma cells inoculated intracamerally in bg/bg and control mice

<table>
<thead>
<tr>
<th>Genotype/ inoculum</th>
<th>Survival, mean days (±SEM)</th>
<th>Frequency</th>
<th>Pulmonary metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/IC 10^2</td>
<td>17.0(±0.9)</td>
<td>3/6</td>
<td>1.3 (±0.7)</td>
</tr>
<tr>
<td>Beige/IC 10^2</td>
<td>16.6 (±1.8)</td>
<td>7/9</td>
<td>30.5 (±11.3)</td>
</tr>
<tr>
<td>Control/IC 10^3</td>
<td>23.0(±1.1)</td>
<td>3/6</td>
<td>2.4 (±2.5)</td>
</tr>
<tr>
<td>Beige/IC 10^3</td>
<td>22.3 (±2.2)</td>
<td>8/8</td>
<td>120.0 (±23.2)</td>
</tr>
</tbody>
</table>

* Inoculum = control (+/+) or beige (bg/bg) mice received intracameral inoculations of either 10^2 or 10^3 B16F10 melanoma cells.
† Mean days = mean survival time as described in Materials and Methods.
‡ Frequency = number of mice with one or more nodules/number of mice per group.
§ Mean number of nodules per mouse.
¶ Apparently healthy tumor-bearing mice were sacrificed.
§ P < 0.01.
Table 3. Effect of LS2616 and cyclophosphamide treatment on survival and metastasis in C57BL/6J mice inoculated intracameraly with 10² in vivo derived B16F10 melanoma cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (±SEM)</th>
<th>Pulmonary metastases (±SEM)</th>
<th>SMLN Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean days</td>
<td>Frequency</td>
<td></td>
</tr>
<tr>
<td>Unenucleated mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>31.1 (±1.6)</td>
<td>0/9</td>
<td>105.6 (±28.6)</td>
</tr>
<tr>
<td>LS2616</td>
<td>35.9 (±1.3)</td>
<td>0/11</td>
<td>51.8 (±15.0)</td>
</tr>
<tr>
<td>Cy</td>
<td>28.9 (±2.0)</td>
<td>0/6</td>
<td>110.9 (±15.1)</td>
</tr>
<tr>
<td>LS2616 + Cy</td>
<td>41.5 (±1.8)†</td>
<td>0/9</td>
<td>26.6 (±7.6)‡</td>
</tr>
<tr>
<td>Enucleated mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E10</td>
<td>33.4 (±2.3)</td>
<td>0/8</td>
<td>122.2 (±25.8)</td>
</tr>
<tr>
<td>E10 + LS2616</td>
<td>44.2 (±2.2)§</td>
<td>2/7</td>
<td>30.1 (±11.9)†</td>
</tr>
<tr>
<td>E10 + Cy</td>
<td>30.8 (±3.1)</td>
<td>0/9</td>
<td>115.8 (±20.4)</td>
</tr>
<tr>
<td>E10 + LS2616 + Cy</td>
<td>62.2 (±2.1)¶</td>
<td>2/9</td>
<td>14.0 (±5.1)¶</td>
</tr>
</tbody>
</table>

* P < 0.05, when compared with untreated controls or Cy alone.
† P < 0.01, when compared with untreated controls, LS2616 alone, or Cy alone.
§ P < 0.05 when compared with E10 + LS2616.
¶ P < 0.01, when compared with E10 or E10 + Cy; P < 0.05 when compared with E10 + LS2616.

had no effect on MPM or frequency of SMLN metastasis (Table 3). Enucleation plus LS2616 treatment resulted in apparent cures. Two of seven (29%) mice remained healthy and were sacrificed and necropsied 11 weeks after enucleation. These mice showed no evidence of metastasis. The group of enucleated and LS2616-treated mice, as a whole, showed a decrease in MPM and frequency of SMLN metastasis and an increase in mean survival time. When enucleated animals treated with LS2616 and Cy combined were examined, the apparent cure rate was 22%, and the group as a whole showed a significant increase in mean survival time. In fact, survival time was the greatest observed in any treatment regimen. MPM was low, and SMLN metastasis was completely prevented (Table 3).

Metastasis of Intracameral Melanoma in PK136 Ab-Treated Mice

To study the effect of marked NK cell depletion on growth and metastasis of ocular tumors, PK136 Ab was administered, as described, to mice inoculated ic with in vivo derived B16F10 melanoma cells. PK136 Ab treatment resulted in the greatest value for MPM ever observed using a 10² tumor cell inoculum (Table 4).

Interestingly, treatment with PK136 resulted in the appearance of extrapulmonary metastases never previously observed in our model of B16F10 murine ocular melanoma (Table 4). Metastasis was observed in adrenal glands (4/10), spleen (4/10) and liver (2/10). In order to examine the effect of PK136 Ab treatment on the LS2616-induced decrease in metastasis from ic tumors, animals were treated with PK136 Ab, given LS2616 as described and inoculated ic with 10² tumor cells. PK136 Ab completely abrogated the decrease in both the MPM and frequency of metastasis to the SMLN induced by LS2616. Moreover, these mice had the same MPM as control untreated mice (Table 4).

Sustained Enhancement or Depletion of NK Cell Activity with LS2616 or PK136 Antibody

In these experiments NK cell activity was assayed in order to ascertain the effect of sustained treatment with either PK136 Ab or LS2616. Panels of three mice were treated with either saline, H16-L10-4 Ab, PK136 or LS2616, sacrificed 7 or 42 days after the start of treatment, and in vitro lysis of 51Cr-labeled YAC-1 target cells measured (Table 5). Mice treated with PK136 Ab and sacrificed at day 7 showed a significant reduction in NK lysis of YAC-1 target cells when compared with saline or H16-L10-4 Ab, PK136 or LS2616, sacrificed 7 or 42 days after the start of treatment, and in vitro lysis of 51Cr-labeled YAC-1 target cells measured (Table 5). Mice treated with PK136 Ab and sacrificed at day 7 showed a significant decrease in NK activity when compared with controls (P < 0.01, Table 5). Mice treated daily for 7 days with 160 mg/kg of LS2616 in drinking water and sacrificed at day 7 showed significantly enhanced NK cell activity when compared to saline treated controls (P < 0.01, Table 5). Mice treated with PK136 Ab and sacrificed at day 7 showed a significant increase in lytic activity when mice treated with LS2616 and sacrificed at day 7 continued to show a significant increase (P < 0.01) in NK activity when compared with controls (Table 5).

The Effects of Treatment with Silica, Carrageenan or Gamma Irradiation on Metastasis of Intracameral Tumors

Silica or carrageenan treatment has been shown by others to be a useful treatment for the reduction of macrophage activity.15-17 In mice inoculated ic with
10^2 B16F10 tumor cells, weekly injections of either silica or carrageenan resulted in MPM of 155.0 ± 62.5/lungs and 191.3 ± 56.3/lungs, respectively. MPM in experimental mice was not significantly different from that observed in controls receiving ic tumor but no drug treatment. Similarly, drug treatment did not appear to affect metastasis to the SMLN. The frequency of metastasis to the SMLN was 8/9 in the controls and 4/6 and 5/6 in the silica and carrageenan groups, respectively (Table 6).

Sublethal gamma irradiation reduces T cell immune responses in the C57BL/6J mouse. In the current study, panels of mice were inoculated ic with tumor cells and irradiated on day 0 (N = 8), day 7 (N = 7) or day 21 (N = 7) postinoculation. No statistically significant differences were seen in the MPM or SMLN when control mice were compared with mice that had received irradiation (Table 6).

**Discussion**

Natural killer cells are large granular lymphocytes with cytolytic activity against a variety of tumor and virus infected cells. NK-deficient mice and rats have been shown to have an increased incidence of spontaneous lymphomas and enhanced growth of metastases from different tumor cell lines. In addition, NK cells may play a role in the regulation of growth and metastasis of human neoplasms. In the current experiments, bg/bg mice and PK136 Ab-treated +/+ mice were used to examine the role played by NK cells in metastasis of ocular tumors of in vivo derived B16F10 melanoma. Monoclonal Ab PK136 is specific for NK1.1 antigen in C57BL/6J mice, and can be used repeatedly in vivo for long-term depletion of NK cells without altering T cell cytotoxicity or humoral immunity. In contrast, anti-asialo GM1 Ab, used in many NK depletion studies, recognizes some macrophage and T cell populations and may induce serum sickness in long-term studies.

Treatment with PK136 Ab resulted in greater than 95% depletion of NK activity (Table 5). In mice bearing ocular tumors, pulmonary metastasis was increased and metastasis was seen in the spleen, adrenal glands and liver (Table 4). This altered pattern of metastasis was striking, as we have previously observed pulmonary and lymph node metastasis with the B16F10 melanoma cell line. These results suggest that NK cell immune surveillance may play a critical role in the regulation of the pattern of metastasis. In comparable experiments, animals harboring flank tumors and depleted of NK cells with PK136 Ab, show occasional extrapulmonary metastasis, but do not demonstrate the pronounced alteration in metastatic pattern observed in mice harboring ocular tumors (manuscript in preparation). We speculate that these differences may be due to the different cell lines used and the different methods of NK depletion.

Table 4. Metastasis of intracranial inoculations of 10^2 B16F10 melanoma cells in NK cell depleted or NK cell enhanced mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean (±SEM)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>H16-L104</td>
<td>33.9 (±2.6)</td>
<td>0/12</td>
</tr>
<tr>
<td>PK136</td>
<td>33.3 (±2.0)</td>
<td>0/7</td>
</tr>
<tr>
<td>H16-L104 + PK136</td>
<td>30.4 (±1.9)</td>
<td>0/10</td>
</tr>
<tr>
<td>LS404 + PK136</td>
<td>38.0 (±2.0)</td>
<td>0/10</td>
</tr>
</tbody>
</table>

*P < 0.01 when compared with untreated controls or LS + PK136.
that NK cells in consort with some feature of anterior chamber-associated immune deviation (ACAID) may determine the pattern of metastasis of ocular B16F10 melanoma.

Examination of metastasis in bg/bg mice showed that these animals had an increase in pulmonary metastasis and a decrease in survival when compared to control +/+ mice. In contrast, survival was not affected in PK136 Ab NK-depleted tumor-bearing mice with ic tumors, even though these animals had a much greater tumor burden in the lungs. In addition, bg/bg mice did not show an altered pattern of metastasis. These differences may be due to the fact that bg/bg mice have decreased T cell functions and decreased macrophage cytotoxicity in addition to having decreased NK activity.

LS2616 treatment activates NK cell activity in C57Bl/6j mice (Table 5). In mice bearing ocular C57BL/6j melanoma.

The results are the mean for three mice ±SEM.

Table 5. Sustained enhancement or depletion of NK cell activity with LS2616 or PK136 Ab treatment

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>100:1</th>
<th>50:1</th>
<th>25:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Saline</td>
<td>14.3 (±1.9)</td>
<td>9.0 (±0.7)</td>
<td>6.3 (±1.7)</td>
</tr>
<tr>
<td></td>
<td>PK136</td>
<td>1.1 (±0.5)†</td>
<td>0.8 (±0.3)†</td>
<td>0.6 (±0.4)†</td>
</tr>
<tr>
<td></td>
<td>LS2616</td>
<td>38.1 (±5.3)‡</td>
<td>24.0 (±4.5)‡</td>
<td>10.1 (±1.5)‡</td>
</tr>
<tr>
<td>42</td>
<td>Saline</td>
<td>17.4 (±1.3)</td>
<td>10.3 (±0.9)</td>
<td>4.1 (±1.1)</td>
</tr>
<tr>
<td></td>
<td>PK136</td>
<td>1.1 (±0.5)†</td>
<td>0.8 (±0.3)†</td>
<td>0.3 (±0.1)†</td>
</tr>
<tr>
<td></td>
<td>LS2616</td>
<td>33.2 (±3.4)‡</td>
<td>18.4 (±2.6)‡</td>
<td>12.1 (±2.1)‡</td>
</tr>
</tbody>
</table>

* Values indicate percent cytotoxicity obtained by killing of YAC-1 target cells in a 51Cr-release assay. Results are the mean for three mice ±SEM.
† P < 0.01 when compared with saline or LS2616.
‡ P < 0.01 when compared with saline or PK136.

Table 6. The effects of silica, carrageenan and gamma irradiation treatment on metastasis of intracameral inoculations of 102 in vivo derived B16F10 melanoma cells in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean days (±SEM)</th>
<th>Frequency</th>
<th>Pulmonary metastases</th>
<th>Mean (±SEM)</th>
<th>Frequency</th>
<th>SMLN Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>31.3 (±1.6)</td>
<td>0/9</td>
<td>105.6 (±29.0)</td>
<td>9/9</td>
<td>8/9</td>
<td></td>
</tr>
<tr>
<td>Silica</td>
<td>34.0 (±3.6)</td>
<td>0/6</td>
<td>155.0 (±62.5)</td>
<td>6/6</td>
<td>4/6</td>
<td></td>
</tr>
<tr>
<td>Carrageenan</td>
<td>37.0 (±2.9)</td>
<td>0/6</td>
<td>101.3 (±56.3)</td>
<td>6/6</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>Gamma irradi†</td>
<td>33.9 (±4.1)</td>
<td>0/7</td>
<td>141.5 (±58.9)</td>
<td>7/7</td>
<td>6/7</td>
<td></td>
</tr>
<tr>
<td>Gamma irradi‡</td>
<td>35.9 (±4.7)</td>
<td>0/8</td>
<td>187.9 (±64.7)</td>
<td>8/8</td>
<td>8/8</td>
<td></td>
</tr>
<tr>
<td>Gamma irradi§</td>
<td>34.8 (±4.5)</td>
<td>0/7</td>
<td>166.0 (±42.7)</td>
<td>7/7</td>
<td>7/7</td>
<td></td>
</tr>
<tr>
<td>Gamma irradi‖</td>
<td>32.0 (±1.6)</td>
<td>0/7</td>
<td>121.4 (±32.5)</td>
<td>7/7</td>
<td>7/7</td>
<td></td>
</tr>
</tbody>
</table>

* Silica injected ip, 1 mg/mouse/week.
† Carrageenan injected ip, 1 mg/mouse/week.
‡ Gamma irradiated B16F10 tumor cells (500 rads).
Lyt-2⁺ lymphocytes in controlling ocular metastasis arising from ic inoculations of B16F10 tumor cells. However, our experiments are not directly comparable. One major difference in our systems is the use of in vivo as opposed to in vitro derived B16F10 tumor cells. In addition, use of cultured cells necessitated the use of irradiated hosts as well as traumatic enucleation of both control and experimental mice in order to obtain pulmonary metastases.

In summary, our experiments clearly demonstrate the importance of the NK cell in regulating metastasis from ocular tumors produced by ic inoculation of in vivo derived B16F10 melanoma cells. In experimental protocols examining the effect of drug therapy on ocular metastasis in tumor-bearing mice, treatment with the NK-enhancer LS2616 in conjunction with low doses of Cy was shown to be effective in decreasing metastasis and increasing survival. An apparent cure rate of 22%, and the greatest control of metastasis and increase in survival, was seen in enucleated mice receiving both Cy and LS2616 treatment.

Key words: ocular melanoma, B16F10, natural killer cells, immune regulation

References