Interferon α2b Decreases Hepatic Micrometastasis in a Murine Model of Ocular Melanoma by Activation of Intrinsic Hepatic Natural Killer Cells

Hua Yang,1 Stefan Dithmar,2 and Hans E. Grossniklaus1

PURPOSE. To investigate in a murine model the mechanism by which micrometastatic melanoma, which spreads from the eye to the liver, is controlled by interferon (IFN)-α2b.

METHODS. Major histocompatibility (MHC) class I antigen (H-2, all haplotypes) expression in three murine melanoma cell lines (Queens, B16LS9, B16F10) was determined by flow cytometric immunophenotyping. The cell lines were heterotopically inoculated into the posterior compartments (PCs) of C57BL/6 mice, and the mice were given intraperitoneal (IP) injections of IFN-α2b or PBS for 1 or 4 days before enucleation at 7 days after inoculation. Groups of mice were made NK deficient or depleted with subcutaneous (SC) injection of anti-asialo GM1. The mice were killed at 28 days or 56 days (survival experiment) after inoculation, and the number of hepatic micrometastases was histologically determined. NK cells were isolated from the spleen and liver at necropsy, and propidium iodide labeled target-specific cytolysis was determined by flow cytometry. The micrometastases were evaluated for apoptosis and proliferation with TUNEL and MIB1 immunostaining, respectively, and TUNEL-to-MIB1 ratios were determined. Hepatic NK cells were immunostained with CD49b.

RESULTS. MHC class I antigen was expressed in the three cell lines in the order of Queens < B16LS9 < B16F10. All cell lines grew, were confined to the PC, and formed hepatic micrometastases. A decrease in micrometastases, an increase in target-specific cytolysis, and an increase in survival correlated with decreased HLA class I expression by the melanoma cells. The IFN-α2b treatment resulted in a boost of intrinsic hepatic NK cells, demonstrated in NK-deficient but not NK-depleted mice. The treatment effect corresponded to increased apoptosis (TUNEL)-proliferation (MIB1) ratios in the micrometastases. Immunostaining demonstrated an increased number of intrahepatic NK cells associated with the micrometastases in treated groups.

CONCLUSIONS. Neoadjuvant IFN-α2b results in decreased hepatic micrometastasis and increased survival time through increased intrinsic hepatic NK cell-mediated tumor apoptosis in a murine model of metastatic ocular melanoma. (Invest Ophthalmol Vis Sci. 2004;45:2056–2064) DOI:10.1167/iovs.03-1331

**MATERIALS AND METHODS**

**Mice**

Twelve-week-old female C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME). All experiments were conducted according to the Declaration of Helsinki and Guiding Principles in the Care and Use of Animals and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Tumors**

B16F10 cells (courtesy of Jerry Niederkorn, UT Southwestern, Dallas, TX) are a line of B16 melanoma cells that were obtained after isolation of metastases and serial passages in tissue culture.13 Queens melanoma cells (courtesy of Jerry Niederkorn) were derived from serial passages of B16F10 melanoma cells.14 B16LS9 cells (courtesy of Dario Rusciano, Friedrich Miescher Institut, Basel, Switzerland) express high levels of c-Met and metastasize to the liver.15 All three cell lines are of cutaneous melanoma origin. All frozen cell lines were thawed and resuspended in 15 mL of minimum essential medium (MEM) supplemented with fetal calf serum, L-glutamine, and sodium bicarbonate. The cell suspension was centrifuged and the pellet was washed and resuspended in 15 mL of supplemental MEM. The suspension was placed in a 75-cm² tissue culture flask (T-75; BD Biosciences, Franklin Lakes, NJ) in a carbon-dioxide incubator (Kendro, Asheville, NC) at 37°C and grown to confluence in 3 to 5 days. The cells were trypsinized, aliquoted, and washed twice in 5 mL Hanks’ balanced salt solution. An aliquot
of 10 μL of suspension was placed in a hemocytometer (AO, Buffalo, NY) to calculate the concentration of melanoma cells.

**Inoculation of Melanoma Cells into the Posterior Compartment**

Aliquots of $5 \times 10^5$ cells/2.5 μL were inoculated into the posterior compartment (PC) of the right eyes using a transcorneal technique that allows the inoculated cells to remain in the eye. Briefly, the mice were anesthetized and a tunnel was prepared from the limbus within the cornea, sclera, and ciliary body to the choroid with a 30-gauge needle under the guidance of a dissection microscope. The tip of a 10-μL glass syringe with a blunt metal needle (Hamilton, Reno, NV) was introduced into the PC through the needle track, and no cells were inoculated until the needle tip was inside the eye. A 5.0-μL suspension of cells was inoculated. No tumor cell reflux occurred, and the subconjunctival space remained free of tumor cells. The right eyes were enucleated 7 days after inoculation. For each experiment, there were 15 mice in each group and each experiment was repeated.

**Interferon**

Recombinant human IFN-α2b (Intron A; Schering-Plough, Kenilworth, NJ) was used. The mice were given 20,000 IU (20KIU) IFN-α2b per intraperitoneal (IP) injection.

**Treated and Control Groups**

There were three groups (4-day IFN-α2b and 1-day IFN-α2b [control]) for each cell line (B16F10, B16LS9, and Queens). The 4-day treatment group received an IP injection of 20KIU IFN-α2b each day for 4 days before enucleation, and the 1-day treatment group received an IP injection of 20KIU IFN-α2b on the day of enucleation.

**FIGURE 1.** PC melanoma model. Heterotopically transplanted melanoma grows in the choroid and subretinal space (A, B) of the PC model and is capable of forming hepatic micrometastases (C). The intraocular tumor is vascularized and metastasizes hematogenously. Hematoxylin and eosin; magnification: (A) ×10, (B) ×25, (C) ×100.

**FIGURE 2.** Flow cytometry to determine MHC-I antigen expression in melanoma cell lines, showing mean fluorescence intensity for MHC-I expression in Queens < B16LS9 < B16F10 (blue) compared with negative control (red). The mean fluorescence intensity for MHC class I for Queens, B16LS9, and B16F10 cells is 0.38, 1.59, and 6.37, respectively (rat anti-mouse H-2, all haplotypes). The x-axis shows side scatter, and the y-axis shows number of cells.
TABLE 1. Number of Hepatic Micrometastases in Uveal Melanoma Mice, with or without IFNα2b Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Queens</td>
</tr>
<tr>
<td>IFN 4 days</td>
<td>1.84 ± 0.26*</td>
</tr>
<tr>
<td>IFN 1 day</td>
<td>2.12 ± 0.81†</td>
</tr>
<tr>
<td>PBS</td>
<td>8.08 ± 1.19</td>
</tr>
</tbody>
</table>

IFN 4 days, IFNα2b IP, injection for 4 days prior to enucleation; IFN 1 day, IFNα2b IP injection for 1 day prior to enucleation.

* Significant difference from PBS control group in the number of hepatic micrometastases (P < 0.01; Wilcoxon Mann-Whitney test).
† Difference from PBS control group in the number of hepatic micrometastases (P < 0.05; Wilcoxon Mann-Whitney Test).

Injection 1 day before enucleation. The control groups received an equal volume of IP PBS every day for 4 days before enucleation. Mice were killed at 28 days after inoculation or observed for survival up to 56 days after inoculation.

**Anti-asialo GM1 NK Deficiency and Depletion**

Two groups (+4 day IFNα2b/anti-asialo GM1 and 4-day PBS/anti-asialo GM1) were used for three cell lines (B16F10, B16LS9, and Queens). Methods for IP injection and PBS were as described earlier. Anti-asialo GM1 (Rabbit; Wako Pure Chemical Industries, Osaka, Japan) was diluted to 1:4 in distilled water. For NK deficiency, the groups were injected subcutaneously (SC) with 100 μL anti-asialo GM1 every 3 days, starting 2 weeks before enucleation and continuing until the mice were killed. For NK depletion, Queens cells only were used in a separate experiment and 100 μL anti-asialo GM1 was injected SC every day starting 2 weeks before enucleation and continuing until the mice were killed.

**Assay for Micrometastasis**

The eyes were routinely processed for light microscopic examination. Serial 5-μm-thick sections were stained with hematoxylin-eosin and evaluated for the presence and location of the melanoma (Olympus BX41; Olympus, Tokyo, Japan). Only mice with melanoma confined to the eye were used in the study. Less than 5% of the eyes had melanoma growing outside the eye. The mice were euthanatized 28 days after inoculation (21 days after enucleation), and necropsies were performed. The livers were grossly examined, submerged in 4% neutral buffered formaldehyde, and processed for light microscopic examination. Three sections through the center of each liver were microscopically evaluated (Olympus) for the presence or absence of micrometastases (<100 μm in diameter), and the average number of micrometastases per section was determined, as previously described.8,11,12

**MHC-I Flow Cytometry**

Major histocompatibility complex (MHC) class I antigen expression for the three melanoma cell lines (B16F10, B16LS9, and Queens) and host natural killer (NK) cell activity were assessed with a cytometer (FACSscan; BD Biosciences, San Jose, CA). Control cells were spleen lymphocytes from normal C57BL/6 mice. For MHC class I expression, rat myeloma monoclonal antibody directed against mouse H-2 (all haplotypes: IgG2a, clone: M1/42.3.9.8; courtesy of Judy Kapp, Emory University, Atlanta, GA) served as positive control. Purified rat myeloma IgG2a (Zymed, San Francisco, CA) served as an isotype-matched negative control antibody. Single-cell suspensions were prepared and washed in buffer (FACS; BD Biosciences) consisting of phosphate-buffered saline (PBS, pH 7.4) with 1% BSA and 0.02% sodium azide. Cells (5 × 10^5) were incubated with primary antibodies for 30 minutes on ice, washed three times, and incubated with fluorescein-conjugated goat affinity-purified F(ab’)_2 fragment to rat IgG (ICN, Aurora, OH) for 30 minutes on ice. Cell suspensions were fixed in 1% paraformaldehyde for analysis.

**NK Cell Isolation and Flow Cytometry**

Isolating lymphocytes from the liver and spleen was performed by exposing and catheterizing the hepatic portal vein with a 24-gauge, 0.8-mm needle. The eyes were routinely processed for light microscopic examination. Serial 5-μm-thick sections were stained with hematoxylin-eosin and evaluated for the presence and location of the melanoma (Olympus BX41; Olympus, Tokyo, Japan). Only mice with melanoma confined to the eye were used in the study. Less than 5% of the eyes had melanoma growing outside the eye. The mice were euthanatized 28 days after inoculation (21 days after enucleation), and necropsies were performed. The livers were grossly examined, submerged in 4% neutral buffered formaldehyde, and processed for light microscopic examination. Three sections through the center of each liver were microscopically evaluated (Olympus) for the presence or absence of micrometastases (<100 μm in diameter), and the average number of micrometastases per section was determined, as previously described.8,11,12

**Table 2. Number of Spleen and Liver CD49b+NK1.1+ Cells in Uveal Melanoma Mice, with or without IFNα2b**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.89*</td>
<td>15.30†</td>
</tr>
<tr>
<td>IFN 1 day</td>
<td>3.90</td>
<td>6.02</td>
</tr>
<tr>
<td>IFN 4 days</td>
<td>5.26</td>
<td>10.30*</td>
</tr>
<tr>
<td>PBS</td>
<td>3.71</td>
<td>4.23</td>
</tr>
<tr>
<td>B16LS9</td>
<td>3.43</td>
<td>8.70*</td>
</tr>
<tr>
<td>B16F10</td>
<td>1.78</td>
<td>3.85</td>
</tr>
</tbody>
</table>

Data are the ratio (%) of spleen and CD49b+NK1.1+ cells. IFN 4 days is defined in Table 1.

* Significant difference between PBS and control groups in the number of hepatic NK cells to hepatic lymphocytes (P < 0.05; chi-square test).
† Difference between PBS and control groups in the number of hepatic NK cells to hepatic lymphocytes (P < 0.01; chi-square test).
Table 3. Number of Hepatic Micrometastases in the IFNα2b–Treated versus Control Uveal Melanoma Mice with Anti-asialo GM1 NK Deficiency

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>Group</th>
<th>Queens</th>
<th>B16LS9</th>
<th>B16F10Q</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IFN 4 days</td>
<td>5.78 ± 1.40*</td>
<td>5.50 ± 2.43*</td>
<td>6.25 ± 2.42†</td>
</tr>
<tr>
<td></td>
<td>IFN 1 day</td>
<td>6.3 ± 1.55*</td>
<td>8.42 ± 2.09†</td>
<td>8.57 ± 5.81</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>14.91 ± 2.06</td>
<td>14.07 ± 3.08</td>
<td>17.83 ± 7.45</td>
</tr>
</tbody>
</table>

Table 4. Percentages of Spleen and Liver CD49b+NK1.1+ Cells in 10,000 Spleen or Liver CD49b+NK1.1+ Cells in 10,000 Spleen or Liver CD49b+NK1.1+ Cells in 10,000 Mice with Anti-asialo GM1 NK Deficiency

<table>
<thead>
<tr>
<th>Groups</th>
<th>Queens</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN 4 days</td>
<td>1.72%</td>
<td>6.30%*</td>
<td></td>
</tr>
<tr>
<td>IFN 1 day</td>
<td>1.56%</td>
<td>3.50%</td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>1.57%</td>
<td>2.62%</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Number of Hepatic Micrometastases in the IFNα2b–Treated versus Control Uveal Melanoma Mice with NK Depletion

<table>
<thead>
<tr>
<th>Group</th>
<th>Queens</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN 4 days</td>
<td>85.40 ± 12.32</td>
</tr>
<tr>
<td>PBS</td>
<td>85.56 ± 15.44</td>
</tr>
</tbody>
</table>

No significant difference from PBS control group in the number of hepatic micrometastases (P = 0.981; Wilcoxon Mann-Whitney test). IFN 4 days, see Table 1. All mice were NK depleted with 100 µL of 1:4 anti-asialo GM1 per day, injected SC starting 2 weeks prior to enucleation until the mice were killed.
merged in dilute streptavidin (DAKO) 1:300 and incubated for 30 minutes. They were washed three times, incubated with 3-amino-9-ethylcarbazole (AEC; Sigma-Aldrich, St. Louis, MO) for 5 to 10 minutes, and counterstained with hematoxylin, mounted in aqueous medium, and coverslipped.

**Apoptosis-Proliferation Rates.** The TUNEL (apoptosis) and MIB1 (proliferation) rates were determined for each group by counting the number and determining the percentage of TUNEL- or MIB1-positive cells in micrometastases in adjacent serial sections of the livers.

**NK Cells.** Frozen sections were cut at 5 μm and mounted on microscope slides (ProbeOn Plus; Fisher Scientific) and heated at 37°C overnight. The sections were fixed in acetone, washed in TBST, and incubated with 3% H2O2 for 10 minutes. Biotin and avidin (Dako) were applied to the slides. The slides were incubated in TBST and blocked with normal rabbit serum for 30 minutes, and 1:25 primary antibody (anti-mouse CD49b; eBioscience, San Diego, CA) was applied. The slides were incubated for 1 hour at room temperature and washed three times, and 1:200 biotin-conjugated anti-Armenian hamster IgG (eBioscience) was applied. The slides were incubated for 30 minutes and washed in TBST. Diaminobenzidine (DAB; Dako) was added, and the slides were incubated for 2 minutes and counterstained with hematoxylin for 1 minute. The slides were dehydrated with graded alcohol, cleared with xylene, and coverslipped.

**Statistical Analysis**

The Wilcoxon Mann-Whitney test was used to determine whether the distribution of number and size of metastatic melanoma differed among combinations of the groups of mice. The number of hepatic micrometastases was compared between the control versus 1-day IFN-α2b treatment versus 4-day IFN-α2b treatment groups. The number of apoptosis (TUNEL+) and proliferating (MIB1+) cells of the treated and control groups were compared by χ2 test.

**RESULTS**

Intraocular melanoma grew and was confined to the eye in 95% of each group for each cell line (Fig. 1). There was no difference in the histologic appearance of the melanoma in the eye after 1 or 4 days of treatment with IFN-α2b. Flow cytometry showed the lowest level of MHC class I expression in the Queens cell line, followed by B16LS9 and B16F10 cells (Fig. 2). The number of hepatic micrometastases in the treated versus control groups is shown in Table 1. There was no statistically significant difference among the number of micrometastases in the control groups. There was a decrease in the number of hepatic micrometastases with IFN-α2b treatment for all three cell lines, with the greatest decrease in Queens followed by B16LS9 then B16F10 cells (Fig. 3). The decrease was statistically significant for all melanoma cell lines in the IFN-α2b 4-day-treated groups (P < 0.01) and the Queens and B16LS9 cell lines in the IFN-α2b 1-day treatment group. Flow cytometry showed the highest level of NK-specific lysis in the Queens melanoma cell line treated with 4- or 1-day IFN-α2b, followed by B16LS9 and B16F10 (Fig. 4). This reached statistical significance for the Queens melanoma cell line (P < 0.01) and corresponded with the in vitro finding of the largest decrease in micrometastasis in the Queen cell line treatment groups, followed by B16LS9 and B16F10 (Fig. 3). The probabilities for decrease in number of micrometastases and percentage of NK lysis in the 4-day IFN-α2b–treated versus control groups were smallest for Queens (0.0051, 0.0083) followed by B16LS9 (0.0093, 0.034) and B16F10 (0.0094, 0.034) cells. Absolute numbers of micrometastases cannot be compared among cell lines, as single lines were used for separate experiments. The experiments were designed to determine differences in absolute numbers in treated versus control groups for a given cell line, not to compare the absolute number of micrometastases among cell lines. Flow cytometry showed increased hepatic NK activity in the 4-day IFN-treated group for all melanoma cell lines (Table 2). It should be noted that the cell lines tested were of murine cutaneous melanoma origin. Uveal melanoma may or may not yield similar results. In addition, IFN-α2b may upregulate MHC class I antigen. Uveal melanoma may or may not yield similar results.
metastasis in 4-day IFN-α2b–treated versus control mice in most of the micrometastases (Fig. 5). The micrometastases had an increased apoptosis (TUNEL)-to-proliferation (MIB1) ratio (Figs. 6, 7) in hepatic micrometastatic melanoma in the 4- and 1-day IFN-treated groups. The apoptosis rates were significantly higher and the proliferation rates were significantly lower \( (P < 0.01) \) in all three cell lines in both the 4- and 1-day IFN-treated groups. There was significantly longer survival in days in the 4-day IFN-α2b–treated groups (Queens, B16LS9, and B16F10 groups survived 47.23, 45.71, and 44.5 days, respectively) versus the control groups (Queens, B16LS9, and B16F10 groups survived 21.93, 23.93, and 25.13 days, respectively, \( P < 0.01; \) Table 6) in all cell lines and in the 1-day IFN-α2b–treated Queens cell line. Necropsies showed hepatic metastases \( (\geq 100 \mu m) \) in all mice that did not survive.

**DISCUSSION**

There is mounting evidence that NK cells are involved in the elimination of human uveal melanoma metastases to the liver. NK cells eliminate cells that do not express HLA class I antigen.\(^{18}\) Immunohistochemical and population-based studies have shown that patients with uveal melanomas that do not express HLA class I antigen have a better prognosis than those with uveal melanomas that do.\(^{19}\) A large percentage of uveal melanomas exhibit a loss of classic HLA class I antigens, allowing for escape from the immunosurveillance of cytotoxic T lymphocytes.\(^{20}\) HLA class I antigen expression correlates with aggressive uveal melanoma cell types.\(^{21,22}\) Metastatic \( (\geq 100 \mu m) \) uveal melanoma that grows from micrometastatic \( (<100 \mu m) \) melanoma to the liver expresses HLA class I antigen, thus allowing for escape from NK cells.\(^{22}\) Shedding of uveal melanoma cells with low expression of HLA class I antigen into the systemic circulation may facilitate their removal by NK cells and prevent development of micrometastases.\(^{19}\) It appears that NK cells eliminate a subset of micrometastases (those with low HLA class I expression) within the liver.

There is experimental evidence, including in vitro and in vivo studies of murine melanoma, that NK cells’ killing of metastatic uveal melanoma to the liver is IL-2 and TGFβ dependent.\(^{23,24}\) Our laboratory\(^{11}\) and others\(^{25}\) have shown that anti-asialo GM1 elimination of NK cells results in an increased number and growth of hepatic micrometastases in murine models using B16-derived melanoma cell lines. In addition, NK cell stimulation with maleic anhydride divinyl ether re-
IFN-α2b is a dimer formed of 166 aa monomers. Each monomer contains five α helices, three 3/10 helices and eight loops. IFN-α2b binds with the cell surface receptor IFN-α/βR and forms a signal complex resulting in antiviral, antiproliferative, and immunomodulatory effects. IFN-α2b enhances NK cell activity in mice.26,27 Injection of mice with IFN-α2b induces results in increased splenic NK cell cytotoxicity against tumor cells.26,27 IP injection of human recombinant IFN decreases the number of pulmonary metastases of IV-injected B16 melanoma cells in mice.28 IFN-α2b also has antiangiogenic properties29 by downregulation of bFGF in tumor cells, resulting in decreased blood vessel density.30 This effect is less important than other effects in our model, as the micrometastases have not developed their own vascular supply11 and maintain a state of equilibrium (dormancy) where apoptosis equals proliferation.31 The proposed mechanism of action of IFN-α2b in our model is enhanced NK melanoma killing through a series of autocrine and paracrine loops (Fig. 8). In our experiments, all three cell lines responded to IFN-α2b therapy in vivo. It is possible that intrinsic hepatic NK cell lysis rates of micrometastases, rather than MHC class I expression by the melanoma cell lines, was the most important factor related to efficacy of IFN-α2b therapy. The importance of intrinsic NK cells for killing micrometastatic melanoma in our

**TABLE 6. Survival Times after IFNα2b Treatment**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cells</th>
<th>B16LS9</th>
<th>B16F10</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>Queens</td>
<td>21.93 ± 6.19</td>
<td>23.93 ± 9.86</td>
</tr>
<tr>
<td>IFN 1 day</td>
<td>Queens</td>
<td>34.8 ± 13.95*</td>
<td>34.79 ± 12.66</td>
</tr>
<tr>
<td>IFN 4 day</td>
<td>Queens</td>
<td>47.23 ± 9.39*</td>
<td>45.71 ± 12.18*</td>
</tr>
</tbody>
</table>

* Significant difference from PBS control group in the number of hepatic micrometastases (P < 0.01; Wilcoxon Mann-Whitney Test).

**FIGURE 7.** Decreased micrometastatic proliferation after IFN treatment. Immunohistochemical stain for MIB1 (anti-Ki-67, protein expressed during cell cycle) in control (A) and IFN 4-day-treated (B) Queens PC model. ABC; magnification, ×100. The brown reaction product shows numerous proliferating melanoma cells in control (A) but not treated (B) micrometastases. There was significantly more proliferation in the control versus the 4- and 1-day IFN-treated groups (graph). Definition of key is provided in Figure 3.

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model was shown. NK-deficient mice had a limited, but present, intrinsic hepatic NK anti-melanoma effect (Table 4), whereas NK depleted mice had no anti-melanoma effect (Table 5).

Our study confirms preliminary evidence that neoadjuvant IFN-γ/H9251.2b decreases hepatic micrometastases from experimental murine uveal melanoma. Flow cytometric analysis showed that the increased NK cells in IFN-γ/H9251-treated versus control groups is a cell-line dependent phenomenon, with decreased MHC class I expression associated with increased efficacy of IFN-γ/H9251 treatment. The percent decrease in micrometastasis, increased NK activity, and increased survival time was greatest in mice inoculated with Queens (low MHC class I) followed by B16LS9 (moderate MHC class I) and B16F10 (high MHC class I) cell lines. Although there may have been a cytotoxic T-cell (CTL) response to MHC class I, which is upregulated by IFN-γ/H9251, this is probably mechanistically less important, in that there is in vitro evidence that uveal melanoma HLA class I expression is downregulated after administration of IFNa.3,2

We found that there continues to be a treatment effect after systemic anti-asialo GM1. Flow cytometry of circulating and intrahepatic NK cells showed increased intrahepatic NK cells in the IFN-α2b treated mice, even after anti-asialo GM1, indicating that intrahepatic NK cells are involved with in vivo killing of micrometastases. Immunohistochemical staining demonstrated and increased number of NK cells associated with hepatic micrometastasis and increased micrometastasis apoptosis-proliferation after IFN-α2b treatment. Our studies show in our murine model that systemic IFN-α2b decreases hepatic micrometastasis from intraocular melanoma by increasing hepatic NK activity and increasing apoptosis in micrometastatic melanoma. This has therapeutic implications when considering combinations of neoadjuvant and adjuvant therapies for patients with primary uveal melanoma.

Acknowledgments

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References


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