Role of Histamine in Natural Killer Cell-Dependent Protection against Herpes Simplex Virus Type 2 Infection in Mice

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Depletion of natural killer (NK) cells in vivo with anti-NK1.1 monoclonal antibody or anti-asialo-GM1 antiserum drastically reduced survival time in Swiss albino mice infected intravenously (i.v.) with herpes simplex virus type 2 (HSV-2). NK cells play a significant role in defense against hematogenously spread HSV-2 and that histaminergic mechanisms regulate antiviral effector functions dependent on NK cells in vivo.

The diamine histamine promotes several human natural killer (NK) cell-related functions in vitro by inhibiting a cell-contact-dependent, suppressive signal delivered by phagocytes (12, 14, 15). This effect of histamine is transduced by H₂-type histamine receptors (H₂R) on monocytes and granulocytes (14). Similar regulatory mechanisms seem to be operative also in vivo, since single-dose treatment of mice with histamine, or with H₂R-specific agonists, augments NK cell-mediated clearance of YAC-1 murine lymphoma cells from lungs and reduces the formation of lung metastases by NK cell-sensitive B16 (F1/F10) murine melanoma cells (13). Treatment of mice with ranitidine, as well as several other H₂R-specific antagonists, augments the number of metastatic foci induced by B16 melanoma cells and markedly reduces NK cell-mediated clearance of YAC-1 cells in vivo (13).

Infection of target cells with viruses of the family Herpesviridae has been used to study interactions of NK cells with virus-infected cells in vitro. Herpes simplex virus type 1 (HSV-1) and HSV-2 infection enhances the susceptibility of target cells to NK cell-mediated cytolysis (3, 7, 10, 18, 24, 27, 28), and NK cells can inhibit the progression of HSV in infected targets (9, 21). With this background information, we chose to study the role of NK cells in mice acutely infected with HSV-2, with special emphasis on the importance of histaminergic mechanisms. Our data indicate that NK cells play a significant role in defense against hematogenously spread HSV-2 and that histaminergic mechanisms regulate antiviral effector functions dependent on NK cells in vivo.

MATERIALS AND METHODS

Animals. Swiss albino mice were obtained from our own breeding colonies at the Department of Virology, University of Göteborg, Göteborg, Sweden. All food, bedding, and cage materials were presterilized. Experiments were conducted with 6- to 10-week-old female mice.

Compounds. Histamine dihydrochloride (Sigma Chemicals), the H₂R antagonist ranitidine hydrochloride (vial purchased from Glaxo), and human recombinant interleukin-2 (IL-2) (specific activity, 3 × 10⁸ biological response modifier units [BRPMU]/mg of protein; Genzyme) were used. All reported IL-2 units are BRPMU; one BRPMU of IL-2 is equal to 2.25 IU. The compounds were readily dissolved in Iscove’s medium. If not otherwise stated, compounds were administered intravenously (i.v.) in the lateral tail vein in 0.5 ml 24 h before the inoculation of virus. Animals treated with corresponding volumes of a vehicle served as controls.

Virus. HSV-2, laboratory strain B4327 (17), was kindly provided by Stig Jeansson (University of Göteborg) and passaged in monolayers of green monkey kidney cells. Plaque titration of the virus was performed as described in detail elsewhere (17). HSV-2 suspended in Eagle’s medium in a total volume of 0.5 ml was inoculated intraperitoneally (i.p.) or into the lateral tail vein (i.v.).

Depletion of NK cells in vivo. NK cells were depleted by treatment with antibodies to NK1.1 (kindly provided by Claes Öhlen, Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden) or anti-asialo-GM1 (anti-aGM1; lot PDG9536; purchased from Wako Chemicals, Neuss, Germany) as described in detail elsewhere (13). Twenty microliters of anti-NK1.1 (10⁻² dilution of hybridoma supernatant) or anti-aGM1 in 0.5 ml was injected into the lateral tail vein.

Statistical evaluation. Either the Student t test or the Mann-Whitney U test was used. All reported P values are two-tailed.

RESULTS

Role of NK cells in HSV-2-induced encephalitis. To investigate the role of NK cells in resistance against HSV-2, NK cells were depleted with anti-NK1.1 or anti-aGM1 24 h before i.v. inoculation of HSV-2. We used adult Swiss albino mice, which are highly susceptible to HSV-2. In these animals, HSV-2 produces a fatal encephalitis within 1 to 4 weeks after systemic virus inoculation (2).

Treatment with anti-NK1.1 or anti-aGM1 on the day before virus inoculation depleted >90% of the NK cells from the spleen. Treatment with anti-aGM1, but not with anti-NK1.1, also slightly reduced the number of lymphocytes with a T-cell phenotype Lyt2⁺, as reported earlier (31). After treatment with anti-NK1.1, <3% of the NK1.1⁺ NK cells were detected in the spleen for approximately 72 h. Pretreatment numbers of NK cells in the spleen (14 to 19% of the gated lymphocytes) were detected 5 to 7 days after depletion. A similar pattern was observed for NK cell-mediated clearance of i.v.-injected, radiolabelled YAC-1 lymphoma cells from lungs, an assay which is commonly employed to measure NK cell function in vivo (11, 26). On day 1 after treatment with anti-NK1.1, the amount of radioactivity remaining 2 h after the inoculation of radiolabelled YAC-1 cells increased more than 10-fold. Pretreatment levels of NK cell-mediated clearance of YAC-1 cells were observed 7 days after treatment with anti-NK1.1 (data not shown).
TABLE 1. Role of route of infection in NK cell-dependent protection against HSV-2 infection in mice by histamine

<table>
<thead>
<tr>
<th>Expt</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>Survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Anti-NK1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>i.v.</td>
<td></td>
<td>13.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Histamine</td>
<td>i.v.</td>
<td></td>
<td>21.6 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>Ranitidine</td>
<td>i.v.</td>
<td></td>
<td>7.8 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Histamine</td>
<td>50 + 40 i.v.</td>
<td></td>
<td>8.9 ± 0.3*</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle</td>
<td>i.v.</td>
<td></td>
<td>11.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Histamine</td>
<td>50 i.v.</td>
<td></td>
<td>17.2 ± 1.4</td>
</tr>
<tr>
<td>3</td>
<td>Vehicle</td>
<td>i.p.</td>
<td></td>
<td>8.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Histamine</td>
<td>50 i.p.</td>
<td></td>
<td>8.7 ± 0.5</td>
</tr>
</tbody>
</table>

* Data are derived from four separate experiments.

a Data are derived from three separate experiments and show the mean survival times ± the standard errors of the means for five to eight animals.

P < 0.01 versus value for respective vehicle-treated controls (Mann-Whitney U test).

P < 0.01 versus value for histamine (Mann-Whitney U test).

The survival time after i.v. inoculation of HSV-2 was consistently shorter in animals depleted of NK cells with either anti-aGM1 (data not shown) or anti-NK1.1 (Table 1). In contrast, depletion of NK cells did not alter survival time in animals infected by the i.p. route (Table 1). Treatment with anti-NK1.1 3 or 5 days after i.v. inoculation of virus did not significantly alter survival time (data not shown).

Protection against HSV-2 infection by histamine. A single dose of histamine, administered on the day before virus inoculation, markedly prolonged survival time after i.v. infection with HSV-2. The effect was dose dependent at histamine doses of 10 to 100 mg/kg of body weight (data not shown). Results of experiments using HSV-2 inocula of different sizes in mice treated with histamine (50 mg/kg) or a vehicle (control) are shown in Table 2. Histamine did not affect survival time in mice depleted of NK cells with anti-aGM1 (data not shown) or anti-NK1.1 (Table 1). Histamine had no effect on survival time in mice inoculated i.p. with HSV-2 (Table 1).

Reduced survival time of HSV-2-infected mice treated with ranitidine. The effect of histamine on survival time after HSV-2 infection was blocked by the specific histamine H2R antagonist ranitidine, indicating that the effect was mediated by H2R (Table 1). Treatment with ranitidine did not affect survival time in NK cell-depleted mice (Table 1). When used as a single agent, ranitidine significantly reduced survival time in HSV-2-infected animals (Table 3).

Synergistic protection against HSV-2 by histamine and IL-2. A synergistic antimetastatic effect of combined treatment with histamine and IL-2 has been demonstrated in the B16 melanoma metastasis model (13). Furthermore, histamine and IL-2 have been shown to synergistically activate human NK cells in vitro (16). To study the effects of combined treatment with IL-2 and histamine in HSV-2-induced encephalitis, mice were treated with a low dose of IL-2 (3,000 U/kg) and/or a suboptimal dose of histamine (10 mg/kg) 24 h prior to virus inoculation. IL-2 alone did not significantly affect survival time at this dose. Histamine alone produced a minor prolongation of survival time. Mice receiving combined treatment with histamine and IL-2 survived significantly longer than mice treated with either compound alone (Table 4).

DISCUSSION

In summary, the data presented in this report suggest that (i) NK cells mediate protection against HSV-2 after i.v. infection but not after i.p. inoculation of virus, (ii) treatment with histamine partially protects mice against HSV-2-induced encephalitis, (iii) the protective effect of histamine is mediated by H2R and requires an intact population of NK cells, and (iv) treatment with the H2R antagonist ranitidine alone reduces survival time after HSV-2 infection.

In spite of the numerous studies showing that NK cells specifically recognize HSV-infected target cells in vitro (3, 7, 9, 10, 18, 21, 24, 27, 28), evidence that NK cells play a major role in defense against HSV-1 and HSV-2 in vivo is scarce. Bukowski and Welsh (5) reported that NK cells failed to protect mice from lethal HSV-1 infection, on the basis of the

TABLE 2. Protection against HSV-2 infection by treatment with histamine

<table>
<thead>
<tr>
<th>Conc of HSV-2 (PFU/ml)</th>
<th>Survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
</tr>
<tr>
<td>7.5 × 10^3</td>
<td>16.8 ± 1.7</td>
</tr>
<tr>
<td>2.5 × 10^3</td>
<td>19.6 ± 1.9</td>
</tr>
<tr>
<td>7.5 × 10^2</td>
<td>23.8 ± 1.2</td>
</tr>
<tr>
<td>2.5 × 10^2</td>
<td>31.0 ± 1.1</td>
</tr>
</tbody>
</table>

* Animals were treated with histamine (50 mg/kg) 24 h before i.v. inoculation of HSV-2 at the indicated concentration.

P < 0.01 versus value for controls.

TABLE 3. Aggravation of HSV-2 infection by treatment with the histamine H2R antagonist ranitidine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative survival time (%)</th>
<th>n</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>100 ± 3.9</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Ranitidine, 40 mg/kg</td>
<td>65 ± 4.7</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ranitidine, 20 mg/kg</td>
<td>78 ± 4.7</td>
<td>14</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Data are derived from four separate experiments.

P < 0.01 versus value for respective vehicle-treated controls (Mann-Whitney U test).

TABLE 4. Synergy between histamine and IL-2 in protection of mice against HSV-2 infection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>i.v.</td>
<td>7.9 ± 1.4</td>
</tr>
<tr>
<td>Histamine</td>
<td>i.v.</td>
<td>12.0 ± 0.9</td>
</tr>
<tr>
<td>Vehicle</td>
<td>i.p.</td>
<td>11.6 ± 0.5</td>
</tr>
<tr>
<td>Histamine</td>
<td>i.p.</td>
<td>12.8 ± 1.6</td>
</tr>
</tbody>
</table>

* Animals were treated with the respective compounds 24 h before inoculation of HSV-2 (7,500 PFU/ml). Histamine was used at 10 mg/kg, and IL-2 was used at 3,000 BRPMU/kg.

P < 0.01 versus value for histamine (Student’s t test).
finding that animals specifically depleted of NK cells were as susceptible to acute infection as control animals. These authors point out the importance of virus-induced interferon, produced by subsets of leukocytes other than NK cells, the major mechanism for first-line resistance against HSV-1 (5). Rager-Zisman et al. showed that transfer of lymphocytes with an NK cell phenotype (aGMI+ NK1.1+) to cyclophosphamide-treated mice induced protection against HSV-1-induced encephalitis (25), indicating that NK cells may participate in second-line defense against HSV-1. The genetic resistance to HSV-2 does not correlate with NK cell levels in mice (1), and mice of the beige genotype, which are deficient in NK cell function, are reportedly not more susceptible to HSV-2 than their coisogenous littermates (23). Kunder et al. did not detect any effect of depletion of NK cells in mice on survival time after infection with a lethal dose of HSV-2 (19, 20).

Such data have been the basis of the present consensus that NK cells do not play a significant role in resistance against HSV-2 in vivo (30, 32). It should, however, be noted that HSV-2 was inoculated by the i.p. route in all of the above-cited studies. Accordingly, we did not observe any effect of NK cell depletion on survival time after i.p. inoculation of HSV-2. On the contrary, animals depleted of NK cells in vivo, with either anti-aGMI or anti-NK1.1, developed encephalitis and died significantly earlier than controls after i.v. injection of virus.

This finding suggests that the route of infection is critical for NK cell-mediated defense against HSV-2. It seems likely that the protective effect of NK cells is confined to early events in viral pathogenesis, since depletion of NK cells 3 days after infection did not alter survival time. A similar pattern has been observed in mice acutely infected with murine cytomegalovirus. In these animals, dissemination of the virus and increased mortality are noted only when NK cells are depleted during the first 3 days after infection (4, 6).

The pathogenesis of i.p.-inoculated HSV-2 in Swiss mice has been described in detail in earlier reports. Replication in peritoneal macrophages is reportedly a major determinant of the outcome of infection (22, 29). Our data showing that i.p.-infected mice are not protected by NK cells suggest that the virus is not accessible to NK cell-mediated protection in this process. That NK cells confer protection after i.v. infection may be clinically relevant, since HSV-2 is spread hematogenously in humans to induce encephalitis in newborns, transplacental congenital infection, and meningitis in adults (8, 33).

The previous observations that histamine augments NK cell cytotoxicity in vivo (13) and the present finding that treatment with histamine significantly prolonged survival time in mice inoculated with HSV-2 support a role for histaminergic mechanisms in the regulation of NK cell function in vivo. Ranitidine blocked the protective effects of histamine, indicating that H2R mediated the response. Furthermore, animals depleted of NK cells were unresponsive to histamine, suggesting that an intact population of NK cells was required for the protective effect. An interesting observation was that ranitidine alone reduced survival time after infection with HSV-2. Similarly, ranitidine effectively reduces NK cell-mediated clearance of YAC-1 cells in vivo and aggravates the metastatic spread of NK cell-sensitive tumors (13). Collectively, these findings indicate that NK cell function, including protection against viral infection, may be dependent on endogenous stores of histamine.

Histamine and H2 antagonists synergistically activate the cytotoxicity of human NK cells in vitro (16), and combined treatment with these compounds prevents formation of pulmonary metastases from B16 melanoma cells in mice (13) and also synergistically augments the clearance of radiolabelled YAC-1 cells from mouse lungs (1a). The observation that a suboptimal dose of IL-2 potentiated the protective effect of histamine in HSV-2 infection in mice further supports the suggestion that histamine and IL-2 synergistically induce activation of NK cell function in vivo.

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REFERENCES


