The Role of Natural Killer Cells in Murine Cytomegalovirus Eye Infection

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Purpose. To study the role of natural killer cells in the dissemination of virus to the eye and virus growth as well as the production of lesions in the eye during primary infection of murine cytomegalovirus.

Methods. Virus activity and lesion production by murine cytomegalovirus in various organs of natural killer cell-depleted BALB/c strain of inbred mice were compared with those of immunocompetent normal BALB/c mice.

Results. In mice injected intraperitoneally with murine cytomegalovirus, virus could be isolated from 50% of eyes in the natural killer cell-depleted group whereas no virus was detected from any eye in the control group. In natural killer cell-depleted mice with positive virus isolation from eyes, no murine cytomegalovirus was isolated from either optic nerves or trigeminal ganglia whereas virus was detected from blood lymphocytes, macrophages, granulocytes, and plasma. After intravitreal injection of murine cytomegalovirus, virus titers in eyes of natural killer cell-depleted mice were significantly higher than those in the control group. Derangement of retinal cell layer and inflammatory cells as well as cytomegalic cells in iris and ciliary body were noted in natural killer cell-depleted group, whereas no such changes were observed in the eyes of the control mice.

Conclusion. Natural killer cell depletion enhances the dissemination of murine cytomegalovirus to the eye through the hematogenous route, and increases virus multiplication as well as lesion production in the eye.

Many ocular diseases caused by cytomegalovirus (CMV) in humans are opportunistic infections and occur predominantly in immunosuppressed individuals. The number of immunosuppressed patients has increased in recent years because of the increased incidence of acquired immunodeficiency syndrome. The profound suppression of immune function in these patients makes them susceptible to many opportunistic agents, such as CMV, and the eye is one of many organs susceptible to CMV infections. Ocular lesions of CMV such as retinitis have been reported in the literature as a manifestation of systemic CMV infection.1,2 Anterior chamber inflammation, uveitis, and vitreous abnormalities have also been reported.3 Furthermore, recent studies indicated that CMV retinopathy is the most common ocular infection in patients with acquired immunodeficiency syndrome.4 The pathogenesis of human CMV eye infections is poorly understood. Some information is available concerning the pathology of CMV-induced human eye lesions, and CMV has been recovered from aqueous and vitreous humor and retinas of human patients.5-8 A few experimental infections of the eye with murine cytomegalovirus would be of interest in studies of human infection.
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NK Cells and Murine Cytomegalovirus Eye Infection

CMV (MCMV) have been carried out in mice, and lesions and virus isolation from the eye have been reported. However, the pathogenesis of the eye infection remains unclear.

The main problems in understanding CMV eye infections concern the mechanism through which the virus travels to the eye from the primary infection site, such as lungs and kidneys, and the effect of immunosuppression on its dissemination to the eye as well as the multiplication of the virus in the eye. Therefore, we assessed the role of cellular immunity, namely natural killer (NK) cells, in the dissemination of the virus to the eye during primary systemic infection of MCMV in mice and virus growth in the eye after primary eye infection.

MATERIALS AND METHODS

Animals

Six-week-old BALB/c strain of inbred male mice from Harlan Sprague Dawley (Indianapolis, IN) were used throughout the study. They were free from MCMV infection as shown by negative antibody against the virus. All animal experiments conformed to the ARVO Resolution on the Use of Animals in Research.

Virus

Smith strain of salivary gland-passed MCMV was obtained from the American Type Culture Collection (Rockville, MD) and maintained in BALB/c mice. Stock virus was a supernatant of salivary gland homogenate of MCMV-infected mice containing 10% dimethylsulfoxide and was stored at −70°C until use. The titer of the stock virus was 10^7 plaque-forming unit (pfu) per milliliter.

Treatment of Mice with Anti-asialo GM1 Serum

To deplete NK cell activity in vivo, anti-asialo GM1 rabbit serum (Wako Chemicals Inc., Richmond, VA) was diluted 1:15 in saline and injected into a tail vein in the volume of 0.2 ml.

Injection of Mice with MCMV

MCMV was injected intraperitoneally with a 23-gauge needle without anesthesia, and each mouse received 0.1 ml of virus suspension containing 2 × 10^3 pfu of MCMV. For intravitreal injection of MCMV, mice were anesthetized with intramuscular injection of both xylazine (5 µg/g) and ketamine (10 µg/g), and 1 µl of diluted virus containing 7 × 10^3 pfu of MCMV was injected into the vitreous cavity through pars plana with 30-gauge needle attached to a Hamilton syringe.

NK Cell Assay

According to the method of Habu et al., YAC-1 target cells were labeled with 100 μCi of sodium ⁵¹Cr (Amersham Corporation; Arlington Heights, IL) for 1 hr at 37°C, washed 3 times with RPMI 1640 medium and seeded in 96 wells (10^4 cells/100 µl/well). Spleen cells, after undergoing hypotonic shock for the elimination of red blood cells, were washed twice with RPMI 1640 and mixed with the target cells in 96 wells (5 × 10^⁵ cells/100 µl/well). Both target cells and spleen cells were suspended in 10% RPMI 1640 containing 10% fetal calf serum. For the determination of spontaneous release, 100 µl of RPMI 1640 with 10% fetal calf serum was added to each well, and for the determination of maximum release, 1% Nonidet P-40 (Sigma Chemical Co., St. Louis, MO) was added. Plates were incubated for 16 hours and centrifugated at 200 X g for 5 min. The radioactivity of 100 µl of the supernatant was measured in a Beckman gamma-counter. The percent of specific release was calculated with the following formula: percent lysis = ([sample release − spontaneous release]/[maximum release − spontaneous release]) × 100.

Analysis of L3T4⁺ Cells, Lyt-2⁺ Cells, and MAC-3⁺ Cells

Spleen cells prepared as outlined earlier were washed twice with phosphate buffered saline, and treated with both phycoerythrin-conjugated anti-L3T4 monoclonal antibody and fluorescein-conjugated anti-Lyt-2 monoclonal antibody (Becton Dickinson, Mountain View, CA). A minimum of 10⁵ cells were analyzed for L3T4⁺ (helper T cells) and Lyt-2⁺ (suppressor T cells) by the use of fluorescein-activated cell sorter (Becton Dickinson, San Jose, CA). The spleen cells were also assayed for macrophages with fluorescein-activated cell sorter after staining with rat immunoglobulin G anti-MAC-3 monoclonal antibody (hybridoma M3/84.6.34., American Type Culture Collection) followed by fluorescein-conjugated anti-rat immunoglobulin G (Boehringer Mannheim Biochemicals, Indianapolis, IN). The percentage of L3T4⁺, Lyt-2⁺ or MAC-3⁺ cells compared to the total number of mononuclear cells in the specimen was then obtained.

Preparation of Blood and Tissues for Virus Isolation

After incising the subclavian artery, 0.2 ml of blood was collected in a syringe containing 5 units of heparin. The heparinized blood was then separated into mononuclear cells and plasma by Ficoll-Hypaque gradient centrifugation. In some experiments, the separated mononuclear cells were further treated to obtain macrophages and lymphocytes according to the method described by Kumagai et al. Granulocytes were separated from the bottom layers of Ficoll-Hypaque gradient, after eliminating erythrocytes by hypotonic shock. Each leukocyte fraction contained 20–30% of cell contaminants. Ten percent tissue homoge-
nate of each organ was also prepared in Eagle's minimum essential medium by grinding tissue with a mortar and pestle, and supernatant after centrifugation at 400 × g for 10 min was used for virus titration.

**Virus Titration**

Serially diluted plasma (0.1 ml) of blood cell suspension or supernatant of tissue homogenate was inoculated onto the monolayer of secondary mouse embryo fibroblast cells in a 24-well plate. After incubation at 37°C for 90 min, minimum essential medium containing 0.3% agarose and 5% fetal calf serum was added (1 ml/well) and further incubated at 37°C in a humid atmosphere of 5% CO2 and 95% air. One week later, cell layers were fixed with formalin and stained with 1% crystal violet, and plaques were counted.

**Interferon Assay**

The method of Su et al was used. Briefly, serial two-fold dilutions of serum specimen were made in 96 flat bottom wells. Each well was then seeded with 4 × 10⁴ L-929 cells. After 24 hours of incubation at 37°C, the cells were washed with minimum essential medium and infected with 10⁵ pfu of vesicular stomatitis virus. Two days later, cells were fixed with formalin and stained with 1% crystal violet, and cytopathic effect was observed microscopically. The reciprocal of the highest dilution of the serum which protected 50% of the cells from cytopathic effect was the titer of interferon.

**Anti-MCMV Antibody Assay**

Anti-MCMV antibody titers were measured by enzyme-linked immunosorbent assay according to the method of Krishna. Briefly, 96-flat-bottom wells of plates were coated with 0.1 ml of antigen prepared from infected mouse embryo fibroblast cells. After 24 hr of adsorption, 1% bovine serum albumin in phosphate buffered saline was added. The plates were then washed, and 0.1 ml of serum with serial twofold dilutions was added to each well. After 2 hr of incubation at room temperature, the plates were washed, and 0.1 ml of 1:2000 diluted horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G antibody (Tago, Inc. Burlingame, CA) was added to each well. After 1 hr more of incubation at room temperature, the plates were washed, and 0.1 ml of substrate solution (o-phenylenediamine [1 mg/ml] with 0.03% hydrogen peroxide) was added. After 15 min at room temperature, the optical density of each well at 492 nm was measured with a multichannel photometer (Uniskan; Labsystems Ltd., Research Triangle Park, NC). The reciprocal of the highest serum dilution showing absorbance value of more than two times that of the normal serum control was considered as anti-MCMV antibody titer.

**Histopathologic Study**

The eyes were fixed in a mixture of ethanol and acetone (1:1) and processed in JB-4 methacrylate (Polyscience Inc., Warrington, PA). Three-micrometer-thick sections were cut, stained with hematoxylin and eosin, and photographed by light microscopy. Two sections each at three different levels were taken from each eye for histopathologic study.

**Statistical Analysis**

Significant group differences were evaluated using Student's t test at P < 0.05.

**EXPERIMENTAL DESIGNS AND RESULTS**

1. Selective Depletion of NK Cells by Anti-asialo GM1 Serum

A group of mice was injected IV with anti-asialo GM1 serum, and another group of mice was injected with saline (control group). Three mice in both groups were killed at various times, and spleen cells from each mouse were used for NK cell assay as well as the assessment of L3T4+, Lyt-2+ and MAC-3+ cells.

As shown in Figure 1, lysis activity of NK cells was markedly depressed as early as 3 days after the injection of anti-asialo GM1 serum (P < 0.01); less than 10% of NK cell activity of control was observed on day 3 and the same degree of suppression was maintained through day 11 after injection of anti-asialo GM1 serum. Conversely, the numbers of L3T4+ cells, Lyt-
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**2+ cells and MAC-3+ cells of anti-asialo GM1-injected mice were almost same as those of control.**

**2. Effects of Depletion of NK Cells on Virus Dissemination to the Eye During Primary Systemic Infection**

A group of 16 mice received anti-asialo GM1 serum injected intravenously, and another group of 14 mice served as a control and received saline injected intravenously. Immediately after, both groups of mice were injected intraperitoneally with MCMV. At various times after virus injection, 3–4 mice were killed, and lungs, salivary glands, eyes, brains, optic nerves, and trigeminal ganglia were excised, homogenized, and titrated for virus infectivity. Also, the blood was collected and then separated into mononuclear cells and plasma by Ficoll-Hypaque gradient. Mononuclear cells were counted and used for infectious center assay. Plasma was used for virus isolation right after separation and was also used for the assessment of anti-MCMV antibody and interferon. Spleen cells from each mouse were used for analyses of NK cells, L3T4+ cells, and Lyt-2+ cells.

**Virus Isolation from the Eye:** As shown in Figure 2, no virus was detected from any eye of control group throughout the entire period. However, from anti-asialo GM1-treated group, MCMV was isolated from 38% (3/8) of eyes on day 6, and from 50% (4/8) of eyes on day 7 ($P < 0.05$), whereas no virus was detected from any eye on day 4 or day 10. The titers of the virus in the eyes ranged from 10 to $2.8 \times 10^2$ pfu/0.1 g of the eye homogenate.

**Virus Isolation from Lungs and Salivary Glands:** Virus titers in lungs of the anti-asialo GM1-treated group were significantly higher than those of control group on days 6 and 7 ($P < 0.01$). The virus titers in the salivary glands in the anti-asialo GM1-treated group were not significantly different from those of the control group (Table 1).

**Virus Isolation from Mononuclear Cells (MNCs), Plasma, Optic Nerves, Trigeminal Ganglia and Brains:** To determine the route by which MCMV travels from the peritoneal cavity to the eye, the rate of virus isolation from optic nerve, trigeminal ganglia, brains, blood MNCs, and plasma of mice was evaluated (Table 2). In our previous experiment, only the mice treated with anti-asialo GM1 serum yielded the virus from the eye and blood. Therefore, only anti-asialo GM1-treated mice with positive virus isolation from one or both eyes were used for the study. MCMV was detected from 50% (7/14) of brains, yet no virus could be detected from optic nerve or trigeminal ganglia of any mouse. Conversely, 86% (12/14) of mice had MNC infected with MCMV (4–75/5 $\times 10^5$ cells) and 100% (14/14) of mice harbored virus in plasma (20–760 pfu/ml).

Interferon and Anti-MCMV Antibody in MCMV-infected Mice: As shown in Table 3, the interferon was detected in low titers on day 4 and became undetectable on day 7 in both anti-asialo GM1-treated and control groups. There were no significant differences in

**TABLE 1. Titers of MCMV in Lungs and Salivary Glands of Mice with Systemic MCMV Infection**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group</th>
<th>Mean Virus Titer (pfu/ml ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 Day</td>
</tr>
<tr>
<td>Lungs</td>
<td>Anti-asialo GM1</td>
<td>190 ± 64</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>70 ± 78</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>Anti-asialo GM1</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not done.

* Significantly higher than control ($P < 0.01$).
TABLE 2. Titers of MCMV in Brains, Optic Nerves, Eyes, and Blood of Mice with Systemic MCMV Infection after Anti-Asialo GM1 Serum Treatment (Days 6 and 7)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;†</td>
<td>ND</td>
<td>&lt;†</td>
<td>300†</td>
<td>23†</td>
<td>140†</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>ND</td>
<td>&lt;</td>
<td>200</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>ND</td>
<td>&lt;</td>
<td>300</td>
<td>40</td>
<td>760</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>ND</td>
<td>&lt;</td>
<td>200</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>ND</td>
<td>&lt;</td>
<td>300</td>
<td>75</td>
<td>540</td>
</tr>
<tr>
<td>6</td>
<td>&lt;</td>
<td>ND</td>
<td>&lt;</td>
<td>2800</td>
<td>35</td>
<td>280</td>
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<tr>
<td>7</td>
<td>&lt;</td>
<td>ND</td>
<td>&lt;</td>
<td>100</td>
<td>5</td>
<td>200</td>
</tr>
<tr>
<td>8</td>
<td>&lt;</td>
<td>ND</td>
<td>&lt;</td>
<td>900</td>
<td>0</td>
<td>140</td>
</tr>
<tr>
<td>9</td>
<td>800</td>
<td>ND</td>
<td>&lt;</td>
<td>50</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>&lt;</td>
<td>&lt;†</td>
<td>&lt;</td>
<td>300</td>
<td>4</td>
<td>60</td>
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<tr>
<td>11</td>
<td>300</td>
<td>&lt;</td>
<td>&lt;</td>
<td>600</td>
<td>8</td>
<td>320</td>
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<tr>
<td>12</td>
<td>&lt;</td>
<td>&lt;</td>
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<td>600</td>
</tr>
<tr>
<td>13</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>200</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>700</td>
<td>&lt;</td>
<td>&lt;</td>
<td>500</td>
<td>32</td>
<td>400</td>
</tr>
</tbody>
</table>

ND = not done.
* Titers of MCMV in both right and left organs were shown.
† pfu/ml of tissue or plasma; < denotes less than 100 pfu (tissue) or 20 pfu (plasma), which are minimal levels of virus isolation.
‡ No. of MCMV-infected cells/5 × 10⁵ MNCs.

Titers between two groups (P > 0.05). Conversely, high anti-MCMV antibody titers were detected early in infection in anti-asialo GM1-treated group (day 4), when the antibody in control group was still below the detectable level (P < 0.01) (Table 4). On day 6 and thereafter, however, no differences were observed in antibody titers between two groups.

NK Cell, L3T4⁺ Cell and Lyt-2⁺ Cell Assay: NK cell activity in control group was 35% on day 3 and 23.4% on day 6, whereas that in the anti-asialo GM1-treated mice was 3.5% on day 3 and 1.4% on day 6; only 10% of NK cell activity of control group on day 3 and 6% on day 6. However, the number of L3T4⁺ cells and Lyt-2⁺ cells in anti-asialo GM1-treated mice were almost same as those of the control at any time.

3. Virus Isolation from Various Cells in Peripheral Blood of Anti-asialo GM1-treated Mice

Mice were injected with anti-asialo GM1 serum and were infected with MCMV as described earlier. Seven days later, when the infection of MNCs with MCMV was most frequent, mice were killed and macrophages, lymphocytes, as well as granulocytes were separated from blood for infectious center assay. As shown in Table 5, MCMV was isolated from lymphocytes in 78% of mice, from macrophages in 75% and from granulocytes in 44%. The mean numbers of infected lymphocytes, macrophages and granulocytes were 17, 151, and 5.2 per 10⁵ cells, respectively.

TABLE 3. Interferon Titer in Plasma During MCMV Systemic Infection

<table>
<thead>
<tr>
<th>Group</th>
<th>4 Day</th>
<th>6 Day</th>
<th>7 Day</th>
<th>10 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-asialo GM1</td>
<td>15 ± 3</td>
<td>5 ± 2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Control</td>
<td>8 ± 0</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

The reciprocal of the highest dilution of the serum producing at least 50% protection of the cells from CPE was the titer of interferon; all values were represented as mean titers ± SD.
### TABLE 4. Anti-MCMV Titer in Plasma during MCMV Systemic Infection

<table>
<thead>
<tr>
<th>Group</th>
<th>4 Day</th>
<th>6 Day</th>
<th>7 Day</th>
<th>10 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-asialo GM1</td>
<td>22 ± 12*</td>
<td>8 ± 5</td>
<td>56 ± 46</td>
<td>1152 ± 644</td>
</tr>
<tr>
<td>Control</td>
<td>&lt;2</td>
<td>16 ± 0</td>
<td>136 ± 92</td>
<td>2304 ± 1288</td>
</tr>
</tbody>
</table>

The reciprocal of the highest serum dilution showing absorbance value at 492 nm of more than two times that of the normal serum control was the titer of anti-MCMV antibody; all values were represented as mean titers ± SD.

* Significantly higher than control (P < 0.01).

### 4. Effects of Depletion of NK Cells on Intravitreal Infection of MCMV

The effects of depletion of NK cells on virus multiplication and lesion production in the eye were studied. A group of 22 mice received a single intravenous injection of anti-asialo GM1 serum, and another group of 21 mice served as a control and received a single intravenous injection of saline. Then MCMV was injected intravitreally to both eyes of mice in both groups. On days 3, 6, 9, and 11 after MCMV injection, mice were killed, and eyes were used for virus titration and histopathologic study.

**Virus Titration of the Eye:** As shown in Figure 3, there were no significant differences in virus titers of eyes between two groups on days 3 and 6. However, on day 9, virus titers in eyes of anti-asialo GM1-treated group (10^3.2 pfu) were significantly higher than those of control group (10^1.3 pfu) (P < 0.01). On day 11, virus titers of both groups were decreased and there was no significant difference between two groups.

### Histopathologic Study:

The anti-asialo GM1-treated group showed derangement of retinal cell layer and thickening choroid with infiltration of many inflammatory cells (Fig. 4) in 50% of eyes (2 of 4) on day 3 and in 75% of eyes (3 of 4) on day 9. No necrotic lesions were noted in the retina at any time, however. Necrotic iris and ciliary body with occasional inclusion bodies were present in 75% of eyes in anti-asialo GM1-treated mice on day 9 (Fig. 4). Conversely, no lesions were seen in

### TABLE 5. Isolation of MCMV-Infected Lymphocytes, Macrophages, and Granulocytes of Mice with Systemic MCMV Infection after Anti-Asialo GM1 Serum Treatment (Day 7)

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Lymphocytes</th>
<th>Macrophages</th>
<th>Granulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.4</td>
<td>0</td>
<td>0.63</td>
</tr>
<tr>
<td>2</td>
<td>0.27</td>
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<tr>
<td>3</td>
<td>0.09</td>
<td>0.39</td>
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<td>0.08</td>
<td>0.09</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0.54</td>
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<td>6</td>
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<td>0</td>
<td>0</td>
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<td>3.9</td>
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<td>8</td>
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<td>960</td>
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</tr>
<tr>
<td>9</td>
<td>100</td>
<td>250</td>
<td>39</td>
</tr>
</tbody>
</table>

**FIGURE 3.** Mice were injected intravenously with either anti-asialo GM1 serum (0.2 ml of 1:5 dilution) or saline (control) and also injected intravitreally with MCMV (7 × 10^2 pfu). They were killed on days 3, 6, 9, and 11 and eyes were excised, 10% homogenates were made and titrated. Each point indicates the MCMV titer in an eye (pfu/0.1 g). The minimum level of virus isolation was 10 pfu/0.1 g of eye tissue. Horizontal lines indicate geometric mean values. There is a significant difference in virus titer between anti-asialo GM1-treated group and control group on day 9 (P < 0.01).
any eyes of control group, throughout the period of investigation.

DISCUSSION

Cellular immunity has been known to affect the infectivity of MCMV. In the mice in which cellular immunity is reduced by either antilymphocyte serum injections, gamma radiation, congenital athymic conditions, or cyclophosphamide injections, MCMV causes disseminating, devastating, and often lethal infection. Conversely, intravenous injection of immune spleen cells to athymic nude mice that were highly susceptible to MCMV resulted in suppression of MCMV infection, but transfer of immune serum to the mice did not affect MCMV infection. These findings suggest that cellular immunity, not humoral immunity, is an important factor for the resistance to MCMV infection.

It has been reported that strains of mice having high NK cell activity were more resistant to MCMV infection than those having low NK cell activity, and that NK cell-deficient homozygous beige mice were more susceptible to the infection than their heterozygous NK cell sufficient litter mates. Also, mice treated with anti-asialo GM1 serum synthesize more MCMV in their livers and spleens and have greater virus-induced liver damage than untreated mice. In our study, anti-asialo GM1 serum treatment did not affect macrophages (MAC-3+ cells), T cells (L3T4+ cells, Lyt-2+ cells), or B cells (anti-MCMV antibody) and effectively lowered only NK lysis activity. Also, anti-asialo GM1 treatment resulted in higher rate of dissemination of MCMV into the eye from the original inoculation site. Therefore, NK cell depletion appears to enhance the dissemination of MCMV to the eye. However, the mechanism by which NK cells affect the dissemination of MCMV to the eye is unclear at present. NK cells can destroy MCMV-infected cells, and it can produce interferon which in turn protects uninfected cells from MCMV infection. However, in our study, there was no significant difference in interferon activity between control group and NK cell-depleted group suggesting that interferon was not an important factor in the prevention of the dissemination of MCMV in our experiments.

The route through which the virus disseminates to the eye from the primary infection site is not clearly understood. In our study, there was a close agreement between the eye and blood of the same mouse in the isolation of the virus, but no such correlation exists between the virus isolation from the eye and that from the brain in the same animal. Furthermore, no virus was isolated from either optic nerves or trigeminal ganglia of any mice. These results indicate that the dissemination occurred primarily through the hematogenous route rather than the neuronal route. Bale et al also reported that after intraperitoneal injection of MCMV, the virus could be recovered from explant of eye on days 3, 7, 14, and 21, explant of optic nerve on days 3 and 21, and from blood on day 3. No attempt for virus isolation from brains was made however.
Therefore, their results have a limited value in evaluating the route of virus dissemination in the mouse. In CMV retinitis in acquired immunodeficiency syndrome patients, hematogenous spread of the virus to the eye was also suspected because CMV was detected from blood6,83 and vascular endothelial cells in choroid6,34 and retina25 of the patients.

MCMV can infect a variety of blood cells, and these cells as well as plasma can serve as virus carriers in the dissemination of the virus to the eye. Our current study indicated that plasma- as well as lymphocyte-, granulocyte-, and macrophage-enriched fractions of the blood of NK cell-depleted mice contained MCMV. Wu and Ho36 have also reported that MCMV was detected in plasma, granulocytes, T cells and B cells in BALB/c mice injected IV with MCMV. Also, macrophages are shown to be permissive to MCMV and to become carriers of MCMV in latently-infected mice.37,38

Immunosuppression enhances the production of lesions in the eye by MCMV. Holland et al11 and Bale et al19 have shown that retinal necrosis developed only in cyclophosphamide-treated mice after intravitreal injection of high titer of MCMV. Also, Mizota et al18 have reported that anterior chamber inoculation of MCMV induced more severe physiologic and morphologic changes in the retinas of severe combined immunodeficient mice than immunocompetent BALB/c mice. However, these studies failed to identify specific immune factors which are associated with pathogenicity of MCMV eye infection. Our study is the first to report that selective immune-depletion of NK cells caused severe lesions and more virus multiplication in the eye following intraocular injection of MCMV than without NK cell depletion. In view of the finding that the enhancing effect of cyclophosphamide treatment on MCMV infection was shown to be related with the depression of NK cells,25 severe histopathologic changes observed in the eye of cyclophosphamide-treated mice reported by previous workers may be due to the depression of NK cell activity by the treatment. However, the exact mechanisms for the enhanced production of lesions and virus growth in the eye in NK cell-depleted mice by MCMV is unclear at present and may warrant further studies.

Key Words

murine cytomegalovirus (MCMV), natural killer (NK) cells, eye infection, hematogenous spread

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References


