Infection of Retinal Neurons during Murine Cytomegalovirus Retinitis

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PURPOSE. Previous results suggest that retinal neurons are infected early during murine cytomegalovirus (MCMV) infection of the inner retina. The purposes of this study were to identify which retinal neurons are infected and to determine the routes by which MCMV spreads in the retina.

METHODS. Immunosuppressed (IS) BALB/c mice were inoculated with 5 × 10^7 PFU of MCMV (k181) through the supraciliary route. Injected eyes were collected at several times after inoculation, sectioned, and examined by electron microscopy and by staining for retinal cell antigens and for MCMV early (EA) or late (LA) antigen.

RESULTS. MCMV-infected cells were observed in the choroid and RPE by day 3 after infection (PI) and in the inner retina beginning at day 5 PI. At this time, many horizontal and bipolar cells were MCMV-antigen-positive but only rare MCMV-infected amacrine cells (glycine positive or γ-aminobutyric acid [GABA] positive) or MCMV-infected ganglion cells (NF positive) were observed in the inner retina. At day 10 PI, most virus-infected cells were glial fibrillary acidic protein (GFAP)- and GABA-positive glia. Virions were observed by electron microscopy in the choroid, RPE, and inner nuclear layer of the retina. Although virions were observed in the endothelium of the retinal vessels and the nearby retinal cells, the endothelial cell lining of the retinal vessels remained intact. Both apoptotic cells and necrotic cells were seen in the inner retina.

CONCLUSIONS. In the inner retina, horizontal and bipolar cells were the early (< day 7 PI) targets of MCMV infection. Virus spread from the RPE and the photoreceptor layer to the inner retina through infected Müller cells and within the inner retina horizontally through infected horizontal cells (Invest Ophthalmol Vis Sci. 2005;46:2047–2055) DOI:10.1167/iovs.05-0005

Cytomegalovirus (CMV) retinitis is the most common sight-threatening opportunistic infection observed in adult and pediatric patients who are immunosuppressed (IS) as a result of chemotherapy, malignancy, or the acquired immunodeficiency syndrome (AIDS).1–4 CMV chorioretinitis, the most frequent ocular abnormality in congenital CMV infection, is observed in approximately 15% of infants with symptomatic infection.5–8

Our laboratory has established a mouse model of CMV retinitis with features resembling those observed in humans with cytomegalovirus retinitis.9–11 In this model, inoculation of murine (M)CMV into IS BALB/c mice through the supraciliary route results in progressive focal necrotizing retinitis.10–11 The choroid and RPE are the initial targets of MCMV infection followed by spread of MCMV to the inner retina by day 5 after infection (PI). Although MCMV-infected, glial fibrillary acidic protein (GFAP)-positive glia are observed in the inner retina, most of these cells are infected late in infection (on or after day 10 PI).12 Therefore, because most of the MCMV-infected cells in the inner retina at the early stage of infection were not infiltrating leukocytes, glial cells, or RPE cells, it appeared that retinal neurons might be the early targets of MCMV infection in the inner retina.13 However, the previous studies did not provide information about early infection of the neurons of the inner retina nor about how virus might spread from the early sites of infection in the choroid and RPE to the inner retina. Electron microscopy and costaining for MCMV antigens and for antigens representing different retinal cells were used in the studies described herein to answer the question of how MCMV spreads from the outer retina to the inner retina during the evolution of MCMV retinitis in BALB/c mice.

METHODS

Virus and Virus Titration

The original stock of MCMV (k181 strain) was the generous gift of Edward S. Mocarski (Stanford University School of Medicine, CA). Virus was prepared from the salivary glands of MCMV-infected BALB/c mice, as described previously.10 Virus stocks were titered by plaque assay on monolayers of mouse embryo fibroblast cells13 and stored at −70°C. The average titer of the virus stocks was between 10^7 and 10^8 PFU/mL. A fresh aliquot of stock virus was thawed and diluted to the appropriate concentration immediately before each experiment.

Mice

Female BALB/c mice 6 to 8 weeks of age (Taconic Inc., Germantown, NY) were used in all experiments. Mice were housed in accordance with National Institutes of Health guidelines and were maintained on a 12-hour light–dark cycle and given unrestricted access to food and water. All ocular injections were performed after the mice had been anesthetized with a mixture of 42.9 mg/mL ketamine, 8.57 mg/mL xylazine, and 1.43 mg/mL acepromazine at a dose of 0.5 to 0.7 mL/kg body weight. The treatment of animals in this study conformed to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research and was approved by the Institutional Animal Care and Use Committee of the Medical College of Georgia.

Experimental Plan

All mice were immunosuppressed by intramuscular injection of 2.0 mg sterile methylprednisolone acetate suspension every 4 days beginning on day −2. This treatment typically depletes ≥93% of the CD4^+ and CD8^+ T cells from MCMV-infected mice, as assayed by flow cytometry of splenocytes.11 IS mice were divided into two groups. On day 9, experimental mice (group 1) were injected via the supraocular route with 5 × 10^7 PFU of MCMV k181 contained in a volume of 2 μL and control mice (group 2) were injected via the same route with an equivalent volume of tissue culture medium. Experimental and control mice were killed on days 3, 5, 7, 10, or 14 PI. Eyes were removed and prepared for immunohistochemistry or electron microscopy.

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Immunohistochemistry

The monoclonal antibody (Ab) to an MCMV early gene product was labeled with FITC (Sigma-Aldrich, St. Louis, MO) or biotinylated (Sulfo-NHS-LC-Biotin; Pierce, Rockford, IL) according to the manufacturer’s instructions. Mouse monoclonal antibody to MCMV late gene products was kindly provided by John Shanley (University of Connecticut Health Center, Farmington, CT). Rabbit antibody specific for GLAST, a Müller cell glutamate transporter was kindly provided by David V. Pow (University of Queensland, Australia). Retinal glial cells (including activated Müller cells and astrocytes) were stained with mouse anti-GFAP (Dako Corp., Carpinteria, CA), or rabbit anti-Go, (Chemicon, Temecula, CA). Mouse anti-Goo (Chemicon) was used to identify both rod and cone bipolar cells. Rabbit anti-calbindin (Chemicon) was used to stain horizontal cells. Rat anti-glycine (kindly provided by David V. Pow) and a rabbit anti-yaminobutyric acid (GABA; Sigma-Aldrich) were used to stain glycinergic and GABAergic amacrine cells, respectively. Mouse anti-neurofilament (NF; Sigma-Aldrich) was used to stain ganglion cells as well as some horizontal cells. Rat anti-Mac-1 (Chemicon) was used to identify macrophages/microglia.

For light microscopy, injected eyes were fixed in 4% paraformaldehyde (Electron Microscope Sciences, Hatfield, PA) for 30 minutes, immersed in 25% sucrose overnight, snap frozen, and sectioned on a cryostat. For staining using mouse-derived primary antibodies (anti-MCMV LA, anti-GFAP, anti-Goo, anti-NF), each antibody was mixed with biotinylated anti-mouse (ARK; Dako Corp., Carpinteria, CA). After 15 minutes, the blocking reagent (ARK, Dako), containing normal mouse serum, was added to the mixture. The mouse immunoglobulin in the blocking reagent bound to the biotinylated anti-mouse antibody that was not bound to primary antibody, thus minimizing reactivity with endogenous immunoglobulin in the specimen. The biotin-labeled primary antibody was then applied to the section. Positive staining was detected with either Texas red-avidin or AMCA (7-amino-4-methyl-coumarin-3-acetic-acid)-avidin (Vector Laboratories, Burlingame, CA).

For cell identification using rat-derived antibodies (anti-glycine and anti-Mac-1), the slides were incubated with anti-glycine or anti-Mac-1 first and then with biotin-labeled anti-rat IgG (mouse serum absorbed; Vector Laboratories). Positive staining was detected using Texas red-avidin or AMCA avidin (Vector Laboratories).

For cell identification using rabbit-derived antibodies (anti-GLAST, anti-calbindin, anti-GABA, anti-GFAP (Chemicon)), the slides were permeabilized; blocked in PBS containing 10% normal goat serum, 2% BSA, and 0.5% Triton X-100; and incubated overnight at 4°C in the primary antibody. After they were washed, the sections were reacted with Texas red or AMCA-labeled anti-rabbit antibody (Vector Laboratories).

In double staining experiments, slides were mounted with antifade medium containing DAPI (Vectashield, Vector Laboratories) and examined microscopically. In triple staining experiments, slides were mounted with antifade medium without DAPI (Vectashield, Vector Laboratories) and examined microscopically. For double staining of MCMV EA and retinal cell antigens, the sections were stained first with retinal cell–specific antibody and then reacted with Texas red-avidin or Texas red-labeled anti-rabbit IgG. The slides were then reacted with FITC-anti-MCMV EA and examined.

For double staining of MCMV EA and lectin (for detection of retinal vessels), the sections were stained first with biotin-labeled anti-EA and reacted with Texas red-avidin. The slides were then reacted with FITC-labeled lectin (Sigma-Aldrich) and examined.

For triple staining of MCMV EA, MCMV LA, and lectin antigens (Goo, NF, or glycine), the sections were stained first with mouse or rat retinal cell–specific antibody and then reacted with AMCA-avidin (Vector Laboratories). After biotin and avidin were blocked in the tissues (Avidin/Biotin Blocking Kit; Vector Laboratories), the sections were reacted with anti-MCMV LA, and the immunolabeling was detected with Texas red-avidin, mounted, and examined. The slides were then reacted with FITC-anti-MCMV EA and examined.

For triple staining of MCMV EA, MCMV LA, and retinal antigens for which the antibodies had been raised in rabbits (GLAST, GABA, calbindin, and GFAP), the sections were stained first with one of the rabbit anti-retinal cell antibodies, and the reaction was developed with AMCA-anti-rabbit IgG. The sections were then stained with anti-MCMV LA and the immunolabeling was detected with Texas red-avidin. Finally, the slides were reacted with FITC-anti-MCMV EA and examined.

For triple staining of MCMV EA, GABA, and GFAP or Mac-1, the sections were stained first with mouse anti-GFAP or rat anti-Mac-1 and the reaction was developed with Texas Red-avidin. The sections were then stained with anti-GABA, and the immunolabeling was detected with AMCA-anti-rabbit IgG. Finally, the slides were reacted with FITC-anti-MCMV EA and examined.

Electron Microscopy

Eyes of experimental and control mice were fixed in 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer overnight at 4°C, washed in cacodylate buffer, postfixed with 4% osmium tetroxide for 1 hour at room temperature, dehydrated in a graded ethanol series, and embedded in resin (Pure Embed 812 mixture; Electron Microscope Sciences). Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope (model 400; Philips, Eindhoven, The Netherlands).

RESULTS

MCMV Infection of Retinal Neurons after Supraciliary Inoculation

To determine which retinal neurons were MCMV infected and when they were infected, the injected eyes of experimental and control mice were double stained for MCMV and retinal cells. The antibodies used to stain retinal neurons included anti-Goo for bipolar cells (Fig. 1A), anti-calbindin for horizontal cells (Fig. 1B), anti-glycine for glycinergic amacrine cells (Fig. 1C), anti-GABA for GABAergic amacrine cells (Fig. 1D), and...
anti-NF for ganglion cells and some horizontal cells (Fig. 1E). The pattern of staining with these antibodies was identical in noninjected and mock-injected BALB/c mice (not shown).

After inoculation of MCMV into the supraciliary space of IS mice, the RPE and choroid were the first sites of virus infection (by day 3 PI) followed by the spread of virus to the inner retina beginning on day 5 PI. Although RPE cells were the initial site of MCMV infection, the photoreceptor cells were not infected, because most of the MCMV-infected cells in the inner retina were observed in the inner nuclear layer and not in the nearby outer nuclear layer (Figs. 2, 3, 4, 5, 6). Double staining for Goa and MCMV indicated that some virus-infected cells in the inner nuclear layer were Goa-positive bipolar cells at days 5 (not shown) and 7 (Fig. 2) PI. Horizontal cells were also infected, and calbindin-positive, MCMV-positive, double-stained cells were observed in the inner retina at this time (Fig. 3). As shown in Table 1, approximately 32.97% and 22.86% of MCMV EA-positive cells in the inner retina were Goa-positive bipolar cells and calbindin-positive horizontal cells, respectively, at day 7 PI. No glycine-positive cells were observed (Fig. 4) and only rare GABA-positive, virus-infected cells were observed in the retina at day 7 PI (Fig. 5; Table 1), which suggested that amacrine cells were not the targets of MCMV infection. Double staining for MCMV EA and NF indicated that NF-positive ganglion cells in the ganglion cell layer were not virus infected (Fig. 6).

At day 10 PI, more virus-infected cells were observed in the retina and there was retinitis involving all layers of the retina as described previously. Although some MCMV-infected, calbindin-positive horizontal cells (Fig. 7 and Table 1) and bipolar cells (Table 1) were still noted in the inner retina, as shown in Figure 8, most virus-infected cells were GFAP- or GLAST-positive glia, as observed previously. At this time, no virus-infected, glycine-positive amacrine cells or virus-infected, NF-positive ganglion cells were observed (not shown), although GABA-positive, virus-infected cells were seen in the remnants of the retina (Fig. 9).

By day 14 PI, the retinal architecture of MCMV-infected areas is destroyed, and glial cells that contribute to formation of a fibrocellular scar are recruited to the retina. Not surprisingly, at this time, most of the MCMV-infected cells were GFAP positive and many were also GABA positive (not shown).

### GABA-Positive, GFAP-Positive, MCMV-Infected Cells

Although at day 10 PI MCMV-infected, GABA-immunoreactive cells were observed in the inner retina, early in infection, most

### Table 1. Percentage of MCMV EA-Positive Retinal Cells of the Total Positive Cells

<table>
<thead>
<tr>
<th>Cell Marker</th>
<th>Day 7 PI</th>
<th>Day 10 PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calbindin (horizontal cells)</td>
<td>22.86</td>
<td>12.71</td>
</tr>
<tr>
<td>GABA</td>
<td>0.77</td>
<td>17.85</td>
</tr>
<tr>
<td>Glycine (amacrine cells)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GLAST (muller cells)</td>
<td>4.48</td>
<td>29.00</td>
</tr>
<tr>
<td>Goa (bipolar cells)</td>
<td>32.97</td>
<td>8.82</td>
</tr>
<tr>
<td>NF (ganglion cells)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are the percentage of infected retinal cells positive for cell marker.
GABA-positive cells were not MCMV infected. In addition, because many GFAP-positive glia were infected with MCMV at day 10 PI and since glycinergic amacrine cells were not infected with MCMV at any time, it appeared that the MCMV-infected, GABA-immunoreactive cells observed late in infection were not GABAergic amacrine cells but were instead another non-neuronal cell type, such as glia. To investigate this possibility, sections of injected eyes were triple stained with antibodies specific for GABA, GFAP, and MCMV EA. At day 10 PI, many MCMV-infected GABA-immunoreactive cells were also GFAP positive, although a few-GABA negative, GFAP-positive MCMV-infected cells were also present (Fig. 10). Some uninfected GFAP-positive glial cells located near areas of MCMV infection were GABA positive (Fig. 10). No GFAP-positive, GABA-positive cells were observed in the eyes of normal mice (not shown) or in the injected eyes of MCMV-infected IS mice at day 5 or 7 PI (Fig. 11). In eyes that had been triple stained for GABA, Mac-1 and EA, no GABA-positive, Mac-1-positive cells were observed at any time suggesting that the GABA-immunoreactive cells were not macrophages/microglia (Fig. 12).

**Spread of MCMV from the Choroid and RPE to the Inner Retina**

After supraciliary inoculation of MCMV, the RPE and choroid are the first sites of virus infection followed by spread of virus to the inner retina beginning on day 5 PI. Because most of the MCMV-infected retinal cells were located in the inner nuclear layer (see Figs. 3, 4, 5, 6), the route by which virus spreads to the inner retina is not clear. Previous studies using this model showed that although a few uninfected RPE cells migrate to the inner retina, most of the RPE cells (MCMV-infected and noninfected) remain in their normal anatomic location throughout the infection.12 This finding suggests that spread of MCMV from the RPE to the inner retina is not likely to result from migration of MCMV-infected RPE cells to the inner retina. Because MCMV infection of the choroid and RPE is associated with apoptosis of overlying retinal cells (especially in the outer nuclear layer),12,18 loss of photoreceptors together with retinal folding could allow direct contact between virus and Müller...
cell processes and/or bipolar cells which would in turn permit virus to spread to the inner nuclear layer, as suggested by the pattern of infection shown in Figures 13 and 14. However, in some eyes, the outer nuclear layer was intact even though MCMV-infected cells were observed in the inner nuclear layer. In the absence of detectable nearby infection, there are at least two possibilities to account for virus spread: One is that infection of horizontal cells allows virus to spread horizontally in the retina, as suggested by the staining pattern shown in Figure 15. Another possibility is that MCMV spreads to the retina from the retinal vessels. To study these possibilities, sections of the eye were double stained with lectin and MCMV EA. As shown in Figure 16, some MCMV-infected cells in the inner retina were located in proximity to the lectin-stained retinal vessels, but double-stained MCMV EA-positive, lectin-positive cells were rarely observed in the inner retina.

Electron Microscopy

To augment the light microscopy studies, sections of injected eyes were stained with uranyl acetate and lead citrate and examined by electron microscopy. On day 5 PI, virus particles were observed in the choroid (Fig. 17) and RPE (Fig. 18). Virus particles and retinitis were then observed beginning on day 7 PI in the inner retina (Fig. 19). The endothelium of the blood vessels as well as the nearby retina in the inner nuclear layer contained virus particles (Fig. 20). Although the endothelium of the vessels in the inner nuclear layer was swollen, the endothelial lining of the retinal vessels appeared to be intact. Apoptotic photoreceptor cells were occasionally observed in the medium-injected eye (not shown). Apoptotic cells were noted in the outer nuclear layer of MCMV-injected eyes as early as day 3 PI (Fig. 21A) and both apoptotic and necrotic cells were seen starting on day 5 PI (not shown) and continuing thereafter (Figs. 21B, 21C). As shown in Figure 21B, apoptotic

Figure 9. Photomicrographs of (A) MCMV EA- and (B) GABA-positive cells (arrows) in the injected eye of an IS BALB/c mouse 10 days after injection of MCMV into the supraciliary space. As shown in the merged image (D), many of the MCMV-infected cells were also GABA positive (arrows). DAPI stain (C).

Figure 10. Photomicrographs of (A) MCMV EA-, (B) GFAP-, and (C) GABA-positive cells (arrows) in the injected eye of an IS BALB/c mouse 10 days after injection of MCMV into the supraciliary space. As shown in the merged image (D), many MCMV-infected, GABA-positive cells were also GFAP positive (arrows). As indicated by the circle (D), some GABA-negative, GFAP-positive MCMV-infected cells were observed in the retina. Some uninfected, GFAP-positive glial cells near areas of MCMV infection were GABA positive (D, arrowheads).

Figure 11. Photomicrographs of GFAP (A) and (B) GABA-positive cells in the injected eye of an IS BALB/c mouse 7 days after injection of MCMV into the supraciliary space. As shown in the merged image (D), no GFAP-, GABA-positive cells were visible. DAPI stain (C).

Figure 12. Photomicrographs of (A) MCMV EA-, (B) Mac-1-, and (C) GABA-positive cells in the injected eye of an IS BALB/c mouse 10 days after injection of MCMV into the supraciliary space. As shown in the merged image (D), there were many MCMV-infected, GABA-immunoreactive cells (arrows) but only a few MCMV-infected, Mac-1-positive macrophages/microglia (circle). No Mac-1-, GABA-positive double-stained cells were observed.
photoreceptor cells, characterized by cell shrinkage and condensation of chromatin and of the cytoplasm were observed in the inner retina. Later stages of apoptosis, characterized by more homogeneous chromatin condensation and increased cell shrinkage were also observed (Fig. 21C). Fragmented nuclei were seen occasionally in apoptotic cells (Fig. 21D). In contrast, necrotic cells exhibited margination of chromatin, and the chromatin was neatly apposed to the inner nuclear membrane. Later, nuclear chromatin of necrotic cells was condensed in a well-delimited, spokelike pattern that was detached from the inner nuclear membrane, and the cytoplasm and organelles also exhibited marked lytic changes (Figs. 21B, 21C).

**DISCUSSION**

There are several descriptions of retinal cell infection during CMV retinitis which together indicate that glia, RPE, and the endothelium of retinal vessels are targets of viral infection. However, previous investigations did not determine whether retinal neurons are targets of CMV infection and if they are, which retinal neurons are infected. The results of the studies presented herein demonstrated that in addition to RPE cells and glia, retinal neurons such as horizontal cells and bipolar cells were also targets of MCMV infection in the inner retina, especially during the early stage of infection. Although virus particles were observed in the endothelium of retinal vessels, loss of the endothelial lining was not observed in the retinal vessels. Furthermore, only rare EA-positive, lectin-positive cells were observed, suggesting that endothelial cells are not a major target of MCMV infection in this model. Many MCMV-infected cells were GABA positive at the late stage of disease (≥ day 10 PI). These infected GABA-immunoreactive cells were not GABAergic amacrine cells but instead appeared to be glial cells, since most these cells were also GFAP positive and since most neurons were infected with MCMV earlier after infection (≤ day 7 PI). In addition, glycinergic amacrine cells were not infected with MCMV. Some uninfected GFAP-positive glia were also GABA positive. GABA is a major inhibitor neurotransmitter in the visual system that is released by retinal neurons. Müller cells strongly express plasma membrane GABA transporters (GATs) and are involved in regulation of GABAergic transmission within the retina by providing a fast termination of GABAergic signaling through their highly efficient GABA uptake from the synaptic cleft and extracellular space. Perhaps this normal transporter function of Müller cells is disrupted by MCMV infection of the retina, resulting in accumulation of GABA in these cells. The observation that Müller cells can express GABA is not without precedent. Immediately after reperfusion of retinal ischemia, a shift of GABA

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**FIGURE 13.** Photomicrographs of (A) MCMV-infected and (B) GFAP-positive cells (arrow) in the injected eye of an IS BALB/c mouse 7 days after injection of MCMV into the supraciliary space, which suggests that one route of virus spread to the inner retina is via MCMV-infected, GFAP-positive Müller cells (arrow in merged image, D). DAPI stain (C).

**FIGURE 14.** Photomicrographs of staining of MCMV antigens (EA: A; LA: B; arrows) and Goa (C, arrow) in the injected eye of an IS BALB/c mouse 7 days after injection of MCMV into the supraciliary space, which suggests that one route of virus spread to the inner retina is via MCMV-infected, Goa-positive bipolar cells (arrow in merged image, D).

**FIGURE 15.** Photomicrographs of staining of (A) MCMV EA and (B) calbindin (arrows) in the injected eye of an IS BALB/c mouse 7 days after injection of MCMV into the supraciliary space. The pattern of staining suggests that virus spreads horizontally in the retina through MCMV-infected, calbindin-positive horizontal cells (arrows in merged image, D). DAPI stain (C).

**FIGURE 16.** Photomicrographs of (A) lectin and (B) MCMV EA in the injected eye of an IS BALB/c mouse 7 days after injection of MCMV into the supraciliary space. As shown in the merged image (D), some MCMV-infected cells in the inner retina were located near lectin-positive cells (arrows), and rare cells in the inner retina were lectin-MCMV antigen-positive. DAPI stain (C).
immunoreactivity from inner retinal neurons to the Müller cells and their processes has been reported. A similar shift has also been observed in the brain, and after ischemia-induced injury in the forebrain, reactive astrocytes express GABA immunoreactivity in the area of ischemia.

MCMV-infected cells were observed mainly near the inner nuclear layer of the inner retina, similar to what has been reported during HCMV retinitis in the eyes of patients.

Although real-time tracing studies cannot be performed, our results suggest that MCMV spreads to the inner nuclear layer through two routes: The first route is by direct infection of Müller cells. In this route, initial infection of the choroid and RPE causes loss of the photoreceptor layer, retina folding, and apoptosis of photoreceptor cells. The cell losses and potential spaces created by these pathologic changes may allow direct contact between virus or virus-infected cells and Müller cells and/or bipolar cells, with subsequent spread of virus to the inner nuclear layer. Once in the inner nuclear layer, virus could...
spreading horizontally by infection of contiguous horizontal cells. The second route is spread of MCMV to the inner nuclear layer through retinal vessels. In these studies, virus-infected cells were observed near retinal vessels where the outer nuclear layer was not folded and which did not otherwise appear to be disrupted. In addition, although virus particles were observed in the endothelium of retinal vessels, the endothelial layer of the retinal vessels appeared to be intact, suggesting that spread of virus from endothelial cells to the nearby retina was not a major source of virus. However, since not all parts of every vessel could be studied, the possibility of isolated disruptions of the retinal vessel endothelium allowing virus to spread from a blood vessel to the retina cannot be ruled out.

Apoptotic cells have been observed during microscopic examination of biopsy specimens of eyes from patients with HCMV retinitis, and we have reported in MCMV-infected mice, that TUNEL-positive, apoptotic retinal cells are not virus infected. The electron microscopy results presented herein provide further evidence that MCMV infection is associated with apoptosis of uninfected neuronal cells. Apoptotic cells were observed in the retina as early as day 3 PI, when only the choroid and RPE were virus positive. Also most of the MCMV-infected cells were in the inner nuclear layer and were not apoptotic, whereas many of the photoreceptor cells in the outer nuclear layer were not virus infected but were apoptotic. Because both apoptotic cells and necrotic cells were observed in the retina during the evolution of MCMV retinitis, it is likely that in addition to necrosis, apoptosis of retinal cells contributes to the pathogenesis of MCMV retinitis.

During MCMV infection of the retina, apoptosis may have dual effects. Apoptosis of uninfected retinal cells may limit virus spread by deleting cells that are susceptible to virus infection. Alternatively, apoptosis may augment retinal damage caused by virus infection and in so doing, allow virus to spread more widely within the retina. Support for the former idea is provided by our results showing that neither viral antigen expression nor viral replication was required for apoptosis in MCMV-infected eyes. Results of studies by Wang et al. using mice inoculated with corona virus via the intravitreal route also suggest that retinal apoptosis occurs in the absence of viral antigen expression and/or viral replication and provide addi-

tional support for the idea that apoptosis of uninfected cells may play a role in limiting virus infection. However, how or when the loss of uninfected retinal cells influences virus infection of the retina remains to be deciphered. In contrast, the results presented herein showing that loss of photoreceptor cells correlated with spread of virus to Müller cells supports the second idea that apoptosis may increase retinal damage by allowing virus to spread more widely within the retina. Therefore, additional studies using mice and cultured retinal cells are needed to define the source and timing of apoptosis-inducing factors in MCMV-infected retinal cells, to determine how apoptosis influences the timing or sites of infection and to determine how the processes of apoptosis and necrosis interact during the evolution of cytomegalovirus retinitis.

References


