ROLE OF RETINAL VASCULAR ENDOTHELIAL CELLS IN DEVELOPMENT OF CMV RETINITIS*

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ABSTRACT

Purpose: Although cytomegalovirus (CMV) retinitis is known to occur in association with retinal microangiopathy in individuals with marked immunodeficiency, glial cells are believed to be the initial target cells in the development of retinitis. Moreover, it has been hypothesized that CMV gains access to the retinal glia because of altered vascular permeability. In an attempt to address the hypothesis, we studied 30 autopsy eyes of AIDS patients with systemic CMV infection, with or without clinically apparent CMV retintis.

Methods: The autopsy eyes were processed in three ways. First, dual immunohistochemical studies were done by using anti-CMV antibodies for immediate early, early, and late antigens. The retinal cell types infected with the virus were then determined by using anti-GFAP, anti-VonWillebrand's factor, neuronal specific enolase, and leukocyte marker CD68. Second, selected eyes were processed for in situ hybridization with DNA probe specific to CMV. Third, an eye with clinically apparent CMV retinitis was submitted for electron microscopic examination.

Results: At the site of retinal necrosis in those eyes with a clinical diagnosis of CMV retinitis, the immunohistochemical, in situ hybridization, and ultrastructural examinations revealed that CMV was present primarily in the Müller cells and in perivascular glial cells. Adjacent to these infected cells, focal areas of positive staining for CMV antigen were seen in the glial cells, neuronal cells, and retinal pigment epithelial cells. At these sites most of the retinal capillaries were devoid of endothelial cells. Few vessels located at the advancing margin of retinal necrosis showed the presence of viral proteins in the endothelial cells.

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Conclusions: The present results indicate that retinal vascular endothelial cells could be the initial target in the development of viral retinitis, with subsequent spread of the infection to perivascular glia, Müller cells, and other retinal cells, including the retinal pigment epithelium.

INTRODUCTION

Disseminated cytomegalovirus (CMV) infection is a known life-threatening complication in patients with advanced human immunodeficiency virus (HIV) infection as well as in immunocompromised organ recipients and patients with immune deficiency resulting from hematologic malignancies.[1-4] Among these entities of immunodeficiency, patients with acquired immunodeficiency syndrome (AIDS) commonly develop CMV infection, particularly when their circulating T-lymphocytes, CD4+ cells, fall below 100.[4-6] In such patients, clinically significant infection is seen primarily in the retina; the reported prevalence of this infection among AIDS patients varies from 10% to 40%.[7]

Recent reports indicate that a significant number of those patients with AIDS who show high copy numbers of human CMV DNA in peripheral blood develop the retinitis.[8,9] Other significant factors associated with development of the retinitis include presence of the glycoprotein B group 2 strain of CMV in the blood and a high circulating HIV load.[10] Although CMV retinitis is seen in eyes with the preexisting retinal microangiopathy often noted in HIV-infected patients, the role of microangiopathy, including cotton-wool spots, in the development of CMV retinal infection is not clear.[11,12]

It is well recognized clinically that the retinitis occurs along the retinal vasculature and that progression of the retinal infection follows the vascular pattern, suggesting that the initial target in the pathogenesis of retinitis could be the retinal vascular endothelium. But histologic studies on CMV retinitis rarely reveal the presence of CMV in the retinal vascular endothelium.[12-16] Moreover, productive CMV infection could be demonstrated readily in the retinal glial cells and retinal pigment epithelial cells.[17,18] In the latter cells, CMV infection begins in the apical membranes and spreads from cell to cell across lateral membranes.[18] Such observations lead to the hypothesis that CMV leaks from the retinal vessels, infects the cells of the inner retina, and subsequently infects the retinal pigment epithelium (RPE).[13,17,18] However, studies in other organs suggest that infected circulating monocytes can adhere to activated vascular endothelial cells, and in such organs, infected vascular endothelial cells are readily detectable.[19]

In the present study, we attempted to delineate the cell types involved in the spread of this virus within the retina, utilizing autopsy eyes of AIDS

patients with and without ocular and/or systemic CMV infection.

MATERIALS AND METHODS

From the files of the A. Ray Irvine, MD, Ocular Pathology Laboratory of the Doheny Eye Institute, Los Angeles, we obtained 30 autopsy eyes of AIDS patients with systemic CMV infection (lung, colon, esophagus, or brain), with or without clinically apparent active CMV retinitis. There were 10 eyes (9 cases; group 1) with CMV retinitis and 20 eyes (11 cases; group 2) without such infection. All 30 eyes were fixed in formalin and processed for routine paraffin embedding. Several serial sections were then obtained from each eye for hemotoxylin-eosin staining, immunohistochemical and immunofluorescence preparation, in situ hybridization technique, and transmission electron microscopic studies.

IMMUNOHISTOCHEMISTRY AND IMMUNOFLUORESCENCE

Following deparaffinization in xylene, the sections were rehydrated for 3 minutes each in 95% ethanol and in water. Each slide was covered with 3% hydrogen peroxide for 5 minutes at room temperature, rinsed with distilled water, and then placed in phosphate buffered saline (PBS) for 5 minutes. A dual immunohistochemical procedure was carried out using Universal DAKO Doublestain Kit system 40 (DAKO, Carpenteria, Calif). After blocking with normal swine serum containing bovine serum albumin in 0.05 MTris buffer for 20 minutes, the slides were incubated at 4°C overnight with a combination of the following primary antibodies: (1) an equal volume mixture of mouse anti-CMV antibodies, immediate early antigen (IE, 1:600) and later antigen (L, 1:600) (Chemicon International Inc, Temecula, Calif), and early antigen (E, 1:30, DAKO, Carpenteria, Calif), with rabbit antihuman von Willebrand's factor (vWF; 1:300, DAKO); (2); anti-CMV antibody mixture (IE, E, and L) with rabbit anti-cow glial fibrillary acidic protein (GFAP, 1:100, DAKO); (3) anti-CMV antibody mixture (IE, E, and L) with rabbit anti-neuron specific enolase (NSE, 1:300, DAKO); and (4) rabbit anti-human vWF (1:300) with mouse antihuman macrophage CD68 (1:100, DAKO). Following a 20-minute rinse with PBS buffer, the slides were covered with a mixture of goat antiserum to mouse immunoglobulins and immunoglobulin fraction of swine antiserum to rabbit immunoglobulins in 0.05M Tris buffer for 30 minutes at room temperature, and then rinsed with PBS for another 20 minutes. The slides were consecutively exposed to solutions containing soluble alkaline phosphatase (APAAP)-immune complex and soluble horseradish peroxidase-rabbit antihorseradish peroxidase (PAP)-complex for two 30-minute periods with an intervening 20-minute PBS rinse. Finally, the slides were separately covered with filtered APAAP (20 minutes) and PAP

(5 minutes) substrate solution, washed with distilled water, counterstained with Mayer's hematoxylin, and mounted for light microscopic examination.

For immunofluorescence, the primary antibody was the mixture of anti-CMV antibodies (IE, E, and L) and antihuman vWF. The secondary antibodies included were goat anti-mouse IgG conjugated with fluorescein (1:100, DAKO) and goat anti-rabbit IgG conjugated with TRITC-labeled streptavidin (1:100, Southern Biotechnology Associates, Inc, Birmingham, Ala). The sections of the globes without clinically apparent CMV retinitis (group 2) were deparaffinized, rehydrated, and processed for conventional immunohistochemical staining. The process used was similar to the above description except that only one primary (anti-CMV) and one secondary antibody were used.

IN SITU HYBRIDIZATION

For in situ localization of CMV nucleic acid, the 10 eyes with clinically apparent CMV retinitis (group 1) were processed in a manner similar to that documented in our previous report, using Enzo Biochem kit (Enzo Biochem, New York).[20] Briefly, the sections were deparaffinized in xylene for 12 minutes with 3 changes; rehydrated in 100%, 95%, 70%, and 50% alcohol and then in distilled water; and digested by proteinase K (0.1 mg/mL) at 37°C for 15 minutes. Following inactivation of endogenous peroxidase with 3% hydrogen peroxide at 37°C for 10 minutes and dehydration in the alcohol, the slides were hybridized with biotinylated CMV-specific probe, made from 2 clones of CMV sequences in the Bam H1 site of pBR 322 plasmid. The insert fragments were 17.2 kb and 25.2 kb, respectively (Enzo Biochem). After hybridization with the probe for 20 minutes at room temperature, the slides were washed with a buffer, incubated with avidin-biotinylated horseradish peroxidase complex (Vector Laboratories, Burlingame, Calif) for 15 minutes, and detected by 3-amino-9-ethyl-carbazole (AEC, Sigma, St Louis, Mo) for 10 minutes. The slides were briefly immersed in hematoxylin for counterstaining.

TRANSMISSION ELECTRON MICROSCOPY

A callote obtained from an eye with clinically apparent CMV retinitis (group 1) was submitted for ultrastructural study. The formalin-fixed tissue was processed for epon embedding, in a manner similar to the previously described technique.[21] Thick sections were stained with toluidine blue, and the selected area was trimmed for ultrathin sections and stained with uranyl acetate and lead citrate. These sections were examined under the elctron microscope (Zeiss, Germany).

For the histopathologic analysis, the hematoxylin-eosin preparations of the CMV-infected retinas could be divided into 3 zones according to the extent of retinal necrosis and disorganization: zone A, an area of the reti-

na in which most of the retinal tissue extending from the nerve fiber layer to the photoreceptors shows the presence of viral inclusions or is destroyed; zone B, an area adjacent to zone A, in which the retinal structure is disorganized, but many well-preserved retinal cells containing viral inclusions remain; and zone C, peripheral to zone B, where the retina is well organized, but occasional individual retinal cells contain CMV inclusions.

RESULTS

CMV infection in the retina was characterized by necrotic changes containing cells exhibiting intranuclear and intracytoplasmic inclusion bodies (Fig 1) and multinucleated giant cells. Such inclusion bodies were also seen in the RPE and optic nerve head. All 10 eyes with the clinical diagnosis of CMV retinitis (group 1) revealed some of these typical changes of CMV disease. Of these 10 eyes, 7 demonstrated an area of full-thickness retinal necrosis, classified as zone A (Fig 1), 9 eyes showed changes characteristic of zone B (Fig 2), and 6 eyes revealed zone C changes. Viral inclusions were seen in the RPE of 5 of the 10 eyes. Two of these eyes also showed inclusion bodies in the optic nerve head, mostly adjacent to the retinal infection. Two of the eyes belonging to this group also showed cytoid bodies.

The histopathologic changes of the retinal vessels were variable and included edema, loss, atrophy, or proliferation of the endothelia; hyaline degeneration of the vessel wall; and luminal narrowing. In zone A, the vessel walls were hyalinized and showed total loss of endothelial lining (Fig 1). In contrast, some retinal vessels at zone B showed perivascular fibrosis, atrophy, or loss of endothelial cells, and other vessels showed prominent endothelial cells. In zone C, the retinal vessels appeared intact with a lining of swollen endothelial cells. In 3 eyes, in zone B the endothelial cells contained intranuclear inclusion bodies. At these sites, perivascularly located retinal cells showed the presence of intranuclear and intracytoplasmic inclusions. None of the 20 eyes in group 2 showed retinal necrosis or inclusions in the retina or in the choroid.

IMMUNOHISTOCHEMISTRY AND IMMUNOFLUORESCENCE

All 10 eyes with clinically apparent CMV retinitis (group 1) showed the presence of viral antigen(s) with the anti-CMV antibody mixture. In these 10 eyes, viral antigens were detected in zone A (9/10), zone B (7/10), and zone C (3/10). In zones B and C, viral inclusions were seen mainly in the inner part of retina, including nerve fiber layer, ganglion cell layer, and inner plexiform layer (Fig 3). These changes were seen in 7 eyes and 3 eyes, respectively, in zones B and C. Some of the retinal cells located in

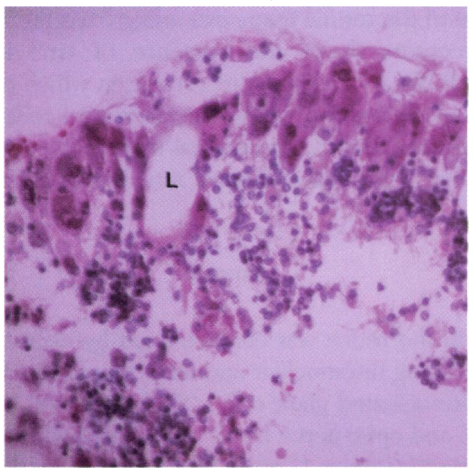

FIGURE 1
CMV retinitis, zone A, characterized by full-thickness retinal involvement by CMV. Note that retinal vessel (L), devoid of endothelium and perivascular and other cells, exhibits intranuclear and intracytoplasmic inclusion bodies (hematoxylin-eosin).

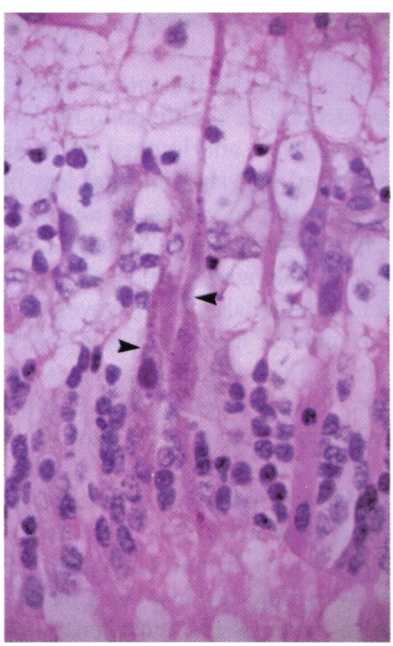

FIGURE 2
CMV retinitis, zone B, shows presence of viral inclusions in Müller cells (arrow; hematoxylin-eosin).

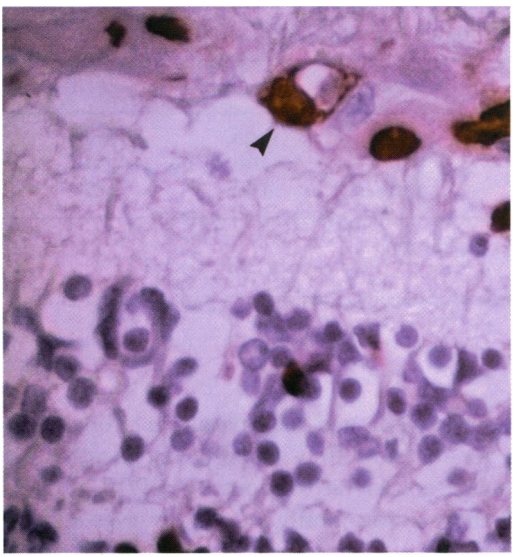

FIGURE 3
Anti-CMV antibodies stain a retinal capillary (arrow) and adjacent glial cells (immunoperoxidase).

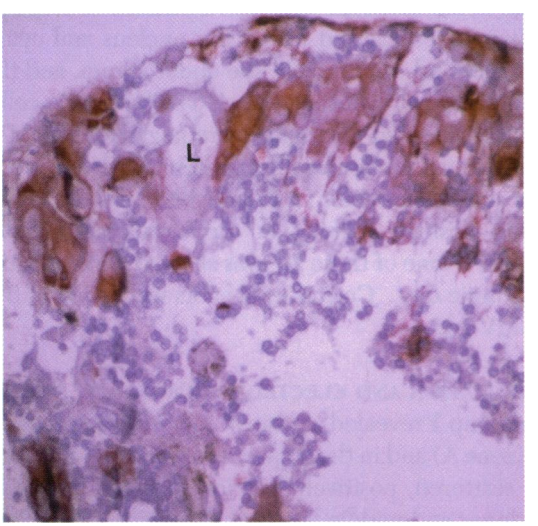

FIGURE 4
Adjacent section of Fig 1 stained with anti-GFAP. Note that several infected cells exhibit positive staining, including perivascular cells (L = vessel lumen; immunoperoxidase).

TABLE I: DETECTION OF CYTOMEGALOVIRUS ANTIGENS BY IMMUNOHISTOCHEMISTRY

RETINA	INNER	MIDDLE	OUTER	RPE
Zone A*	9/10	9/10	9/10	4/10
Zone B	7/10	4/10	2/10	1/10
Zone C	3/10	1/10	0/10	0/10

RPE, retinal pigment epithelium.

*In zone A, full-thickness retinal necrosis suggests involvement of inner, middle, and outer retina.

the inner nuclear layer (INL) and outer plexiform layer also revealed positive staining (4/10 in zone B and 1/10 in zone C). An occasional positive cell was seen in the outer nuclear layer (2/10 in zone B and 0/10 in zone C). RPE staining for the viral antigens was mostly seen in the eyes at the site of zone A (4/10). These immunohistochemical findings are summarized in Table I.

The immunohistochemical stains revealed the presence of viral antigen frequently in Müller cells or astrocytes (Figs 4 and 5). Of the 10 eyes, 9 revealed the presence of viral antigen in these cells. The dual immunofluorescence preparations showed the presence of CMV antigens in vascular endothelia (Fig 6) in 4 eyes, mostly at zone B. In 4 eyes, CMV antigens were seen in the neurons. The distribution of viral antigens varied in these cells; they were localized within the nucleus and cytoplasm of the infected astrocytes and Müller cells, the endothelium, and the RPE. The neuron staining was mainly intranuclear. CD68-positive cells, which represented macrophages and microglia, were negative for CMV inclusions.

Of the 20 eyes without clinically apparent CMV retinitis (group 2), 1 eye showed positive staining for CMV antigen in the retina. Of this group, 3 eyes showed positive staining in the circulating leukocytes of the choroidal vessels. None of these 20 eyes showed CMV-positive staining in RPE or in the optic disc. Cotton-wool spots were seen in 5 eyes that did not display CMV antigens.

IN SITU HYBRIDIZATION AND ELECTRON MICROSCOPY

All 10 eyes in group 1 revealed a positive hybridization reaction within the necrotic foci (zone A) and in the adjacent retina (zone B). In zone B, there were several scattered, positively hybridized individual cells, noted primarily in the inner and middle layers of the retina. Only 2 of the 10 eyes showed positive hybridization reaction within RPE. The ultrastructural examination confirmed the presence of electron-dense particles in the nucleus and cytoplasm of retinal cells. Morphologically, these particles (Fig 7) exhibited features consistent with the herpes group of viruses.[1]

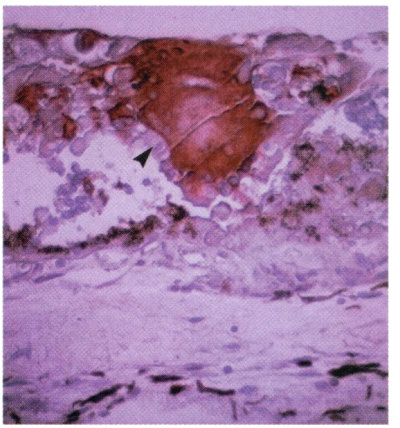

FIGURE 5
CMV retinitis, zone A. Note that multinucleated giant cell stains positive with anti-GFAP antibody (arrow; immunoperoxidase).

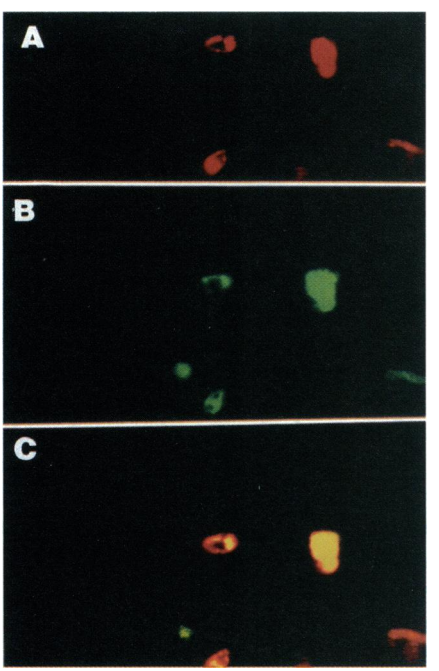

FIGURE 6
CMV retinitis, zone B. Immunofluorescence preparation showing staining of retinal vascular endothelium with anti-human von Willebrand's factor (A). Note positive staining with anti-CMV antibodies at same sites (B). Combined stains reveal orange-yellow staining indicating presence of CMV antigen in the vascular endothelium of several blood vessels (C) (dual immnofluorescence).

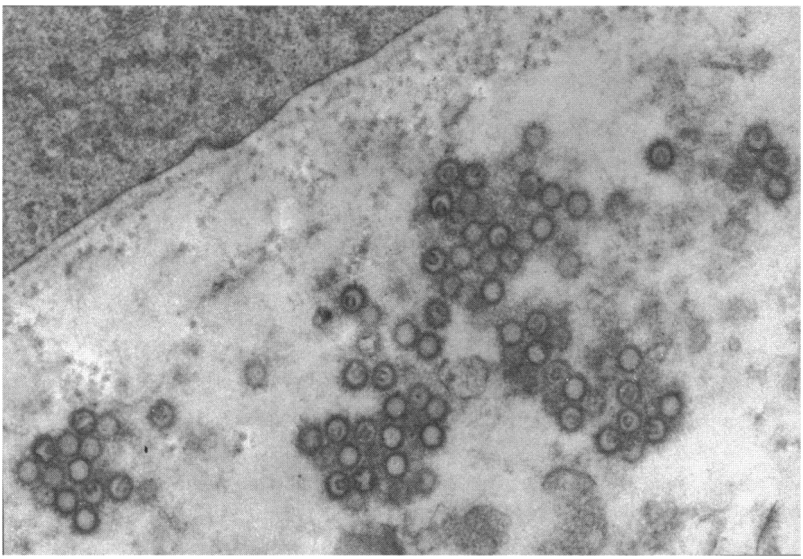

FIGURE 7
Electron microscopic examination of the retina revealed double-walled, viral particles in cytoplasm of infected cell (x44,500).

DISCUSSION

In the current study, immunohistochemical preparations revealed the presence of CMV viral proteins in the retinal vascular endothelium at the sites (zone B) peripheral to the areas of full-thickness retinal damage and necrosis. This zone (zone B) clinically could represent the advancing margin of the viral retinitis. In contrast, the necrotic sites (group A) showed the presence of viral inclusions primarily in the glial cells (Fig 1). At these sites the retinal capillaries and other vessels were devoid of endothelial lining. These findings suggest that the loss of endothelium at the sites of well-established retinitis (zone A) could be due to necrosis and dislodgment of the infected endothelial cells, since such cells are exposed to circulation, whereas other infected retinal cells, which are not directly exposed to the circulation, could remain in the retina. This could explain the easy detection of the viral inclusions in the perivascular astrocytes and Müller cells in this viral retinitis.

Circulating infected vascular endothelial cells that have been detected in patients with CMV infection are believed to be caused by dislodgement of infected vascular endothelium.[19] The absence of endothelial cells in the vessels located in zone A could be the result of a similar phenomenon. Moreover, dislodgement of such infected cells could disseminate the virus

within the retina, leading to the development of multifocal retinal lesions, particularly at the advancing margin of retinitis. Clinically, it is well known that multiple tiny opacities can be seen in the retina at the advancing margin of retinitis. These could represent infected foci of retinal cells from dissemination of the virus from the infected and dislodged endothelial cells. Such retinal changes could be caused by dissemination of the virus from circulating infected leukocytes; but there is no histologic evidence for such leukocytic dissemination in the retina, since none of the monocytes or macrophages (CD68-positive cells) in the retina showed the presence of CMV inclusions. The multifocal nature of the retinitis, with normal-appearing retina between the tiny lesions (at the advancing edge of the retinitis), also suggests that such lesions could be due to hematogenous rather than direct lateral spread of the virus from cell to cell.

It is well recognized that CMV is carried to distant organs by the hematogenous route within the leukocytes, particularly circulating monocytes and other bone-marrow-derived cells. It is conceivable that infected circulating monocytes could attach to retinal vascular endothelium in a host with defective immunity. In an HIV-infected person, the presence of various infections could lead to high levels of circulating cytokines, such as TNF-α and others. Such cytokines are known to upregulate various adhesion molecules in the vascular endothelium.[22-24] Infected, particularly activated, monocytes can readily adhere to the vascular endothelium expressing the adhesion molecules. Once attached to the retinal vascular endothelium, the CMV can spread from monocytes to the vascular endothelium.[25]

Because of the close anatomic relationship of pericytes and astrocytes with the vascular endothelium, it is tempting to speculate that the virus from the infected endothelium could spread to the glial cells (astrocytes), where the virus can thrive and spread to other glial cells including Müller cells. These infected glial cells could spread the virus to neuronal cells and RPE. The present histopathologic study shows the presence of viral inclusions or protein in the glial and Müller cells, including the RPE. The infection of RPE was seen only in eyes with extensive retinal involvement, particularly at the sites (zone A) showing outer retinal infection. Such findings suggest that RPE infection is secondary to Müller cell infection and that the infection spreads not only laterally to other glial and neuronal cells, but also toward the RPE from the inner retina.

The present study also showed the presence of infected cells in zone B and zone C in the inner retina (Table I), particularly around the retinal vessels. Such findings suggest that spread of the infection in the inner retina could initially occur from vascular spread, with subsequent involvement of the deeper avascular retina and RPE by cell-to-cell spread. It appears

that astrocytes/Müller cells can harbor a productive CMV infection,[17] and once infected, can spread the virus to adjacent neuronal cells, including RPE, through extensions of the cell processes. Moreover, these glial cells could play a role in the expansion of infection in the retina, similar to the role played by fibroblasts in other solid organs.[19]

In situ molecular hybridization and ultrastructural examinations confirmed the presence of viral DNA and particles in both the nucleus and the cytoplasm of infected glial and other cells. The glial cells readily demonstrate the presence of viral proteins, nucleic acid, and viral particles in the nucleus and cytoplasm, indicating a productive infection in these cells, as shown by immunohistochemical stains (Figs 3 and 6). Similarly, the presence of viral proteins in the cytoplasm of infected retinal vascular endothelial cells suggests that these cells can be productively infected by CMV and that these infected cells could transmit the virus to adjacent perivascular cells. In preliminary in vitro studies, we could readily infect cultured human retinal vascular endothelial cells exposed to low levels of endotoxin with the laboratory strain of AD169 human CMV. These preliminary findings and current immunohistochemical studies support the hypothesis that CMV infection in the retina initially takes place in the vascular endothelium.

SUMMARY

Our histologic and immunohistochemical studies establish the presence of CMV in retinal vascular endothelium, particularly at the presumed advancing edge of the retinal infection, and the lack of such cells in the established necrotic sites of retinal infection. On the basis of the anatomic and histologic distribution of CMV infection in the retina, it appears that the virus first gains access to the vascular endothelium and subsequently spreads to the perivascular glia and other retinal cells, including RPE.

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DISCUSSION

DR DOUGLAS A. JABS. Cytomegalovirus (CMV) is a ubiquitous herpes family virus. Seroprevalence studies have suggested that 50% of the general population harbors CMV in a latent state and that more than 95% of populations at high risk for human immunodeficiency virus (HIV) infection harbor latent CMV.[1,2] CMV disease is associated with immunosuppression, either in the transplant situation or due to the acquired immune deficiency syndrome (AIDS). The presumed pathogenesis of CMV disease is as follows: With immune suppression or immunodeficiency, latent CMV reactivates into a productive infection. CMV then is hematogenously disseminated to the target organ. Once the target organ is infected, viral replication occurs in the target organ's tissues, resulting in tissue damage. Evidence supporting this hypothesis includes the occurrence of positive blood cultures for CMV in patients with CMV disease[3] and the predictive value of elevated CMV viral load in the blood for the subsequent development of CMV disease.[4] Careful pathologic studies in other organs (eg, the lung) have demonstrated that endothelial cells play a key role in this infection. Vascular endothelial cells are infected initially, with subsequent transmission into target organ tissue.[5]

There are substantial differences in the incidence of CMV retinitis between the transplant situation and AIDS. CMV retinitis is uncommon in patients who undergo transplants, whereas it is common in patients with AIDS. The proportion of patients developing CMV disease after a bone marrow transplant is less than 1%, despite a high incidence of CMV pneumonitis in these patients,[6] but the cumulative lifetime incidence of CMV retinitis in patients with AIDS, prior to the use of highly active antiretroviral therapy, was 30%.[3,7] This difference in the incidence of CMV disease between the transplant situation and AIDS has led to speculation as to possible differences in pathogenesis. One hypothesis has been that HIV retinopathy leads to a breakdown in the blood-ocular barrier, which permits virus to enter the retina more easily and establish a productive infection. Epidemiologic studies have suggested that there is an increase in the probability of CMV retinitis in patients who have HIV retinopathy.[3] Other hypotheses have included the possibility of preexistent or latent infection in the retina.

Dr Rao and colleagues, in this paper, have performed a careful histologic evaluation of CMV retinitis and used several techniques to detect CMV. They have classified the retina into three zones: one where there is full-thickness retinal destruction, one at the edge of full-thickness retinal destruction, and one where there is little evidence of retinal damage. Where there is full-thickness retinal destruction, they demonstrated that retinal vascular endothelial cells have been lost. In contrast, at the edge of

the area of full-thickness retinal destruction and occasionally in areas of normal-appearing retina, there is evidence of retinal vascular endothelial cell infection by CMV. Furthermore, at the edge or in normal retina, CMV infection is confined largely to the inner retina and not present in the outer retina. Infection of retinal pigment epithelial cells can be demonstrated primarily in those areas of full-thickness retinal destruction and are not evident in normal-appearing retina. These results suggest that CMV is transmitted to the retina via the retinal vasculature and that endothelial cell infection is, at least, one mechanism by which retinal infection and disease occurs.

The suggestion that satellite lesions are due to hematologic spread through the retina, although possible, cannot be confirmed by the available data. Because most of the spread of retinitis in patients who were not treated for CMV retinitis occurs into contiguous retina from a preexisting focus rather than to a new site, it would appear that the spread from infected retinal tissue to adjacent retinal tissue is the more likely mechanism. Furthermore, satellites are typically present at the border of the lesion as the lesion progresses into adjacent retina.

In conclusion, this paper demonstrates that infection of retinal vascular endothelial cells by CMV occurs, plays a role in the development of CMV retinitis, and is at least one mechanism by which CMV enters the retina. It also demonstrates that the retinal pigment epithelium has little role in the development of CMV retinitis, and that infection of the retinal pigment epithelium occurs only as a consequence of spread from necrotic retina.

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W. RICHARD GREEN, MD. I would like to ask if you have other data which you didn't present to refute or to confirm the previous study by Newsome and associates who observed immunoprotein in retinal vessel walls?

JOSE PULIDO, MD. Our previous studies had shown that cells from the neurosensory retina were more susceptible to infection by CMV than retinal pigment epithelial cells in vitro. We were pleased to see that Dr Rao now confirms that in vivo the neurosensory cells are infected before the RPE and that infection of the RPE is only a late manifestation of this disease.

NARSING RAO, MD. First of all I would like to thank Dr Jabs for his review of our manuscript and raising an issue pertaining to satellite lesions in CMV retinitis. Clinically, tiny satellite lesions are observed at advancing edge of the retinitis. Based on our histopathologic studies, zone B and C represent advancing edge of the retinitis and at these sites we noted retinal capillaries showing CMV infected endothelial cells and presence of viral particles in the glial cells located around these infected capillaries. We believe these angiocentric lesions could be satellite lesions seen clinically in CMV retinitis. Presence of such angiocentric lesions suggest that intraretinal spread of the infection in the form of satellite lesions could be from initial vascular endothelial infection, followed by infection involving perivascular glial cells, Müller cells and subsequent spread of the infection to RPE.

Dr Green raised an interesting question on the role of immune deposits in the pathogenesis of retinitis. We have not examined the eyes for such immune deposits including immune-complexes. However, our immunofluorescence and immunoperoxidase studies show presence of viral antigen(s) in the retinal vascular endothelium.

Dr Pulido is one of the first investigators to show the importance of retinal glial cells as the target cells in CMV retinitis and pointed out that RPE cells are not the primary target cells in the development of human CMV retinitis.