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Case report

Evidence for cytomegalovirus and human immunodeficiency virus infection of the retina in AIDS

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Summary. Characteristic ophthalmopathological features of retinal lesions in a patient with the acquired immunodeficiency syndrome (AIDS) are reported. In situ hybridization, immunohistochemistry and electron microscopy revealed severe unilateral cytomegalovirus (CMV) retinitis. The opposite retina which was not involved by CMV showed nonspecific signs of ischaemia in the nerve fiber layer corresponding to cotton-wool spots. Occasional cells of both retinas were positively stained by a mouse monoclonal antibody to the p24 HIV-1 antigen indicating infection of retinal cells by HIV. It is suggested that HIV may directly or indirectly damage retinal tissue and interact with opportunistic pathogens, thus leading to a variety of ocular abnormalities associated with AIDS.

Key words: Human immunodeficiency virus – Opportunisitc ocular infections – AIDS-associated retinopathy

Introduction

Severe human immunodeficiency virus (HIV) infection is often accompanied by ocular disorders. CMV retinitis is an increasingly frequent problem in patients with AIDS and may even be an initial manifestation of the profound compromise of the immune system (Henderly et al. 1987a). Several reports document CMV retinitis in 12% to 46% of patients with AIDS or AIDS-related complex (Fay et al. 1988). Retinal lesions are usually characterized by white areas of retinal necrosis and haemorrhagic exsudates in a vascular pattern of distribution ultimately leading to blindness (Henderly et al. 1987a).

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In addition to viral, fungal and parasitic infections of the eye, a noninfectious AIDS-associated retinopathy of presumed ischaemic origin has been noted in more than 50% of patients with AIDS or AIDS-related complex (Freemann et al. 1984; Holland et al. 1982; Mansour et al. 1988; Pepose et al. 1985). The hallmark of this condition is the presence of cotton-wool spots indicating areas of focal retinal ischaemia. Microaneurysms and perivasculitis are also considered as part of noninfectious AIDS-retinopathy (Kestelyn et al. 1985; Newsome et al. 1984).

We performed a postmortem histopathologic examination of the eyes from a patient with AIDS who was found to have CMV retinitis on his left eye and cotton-wool spots on his right eye. Our study suggests that HIV infection may be implicated in the pathogenesis of both CMV retinitis and AIDS-associated retinopathy.

Case report

The patient was a 44-year old homosexual man with a two-months history of visual field loss on his left eye. Three years earlier, he was found to be HIV-1 positive. Fundoscopic examination disclosed retinal necrosis and haemorrhagic exudates in the left eye indicating CMV retinitis. Cotton-wool spots were present in the right retina. Treatment was initiated with ganciclovir but toxicity evidenced by neutropenia required discontinuation of therapy. He died two weeks later.

The postmortem examination revealed central nervous system toxoplasmosis and CMV infection, CMV adrenalitis and Kaposi's sarcoma of the throat.

Materials and methods

Both eyes were removed at autopsy. For light microscopy and immunoperoxidase staining samples from both eyes were embedded in paraffin. Sections were cut at 5 µm. The following stains were used: haematoxylin-eosin, periodic acid-Schiff, Prussian blue, Gomori's methenamine silver stain for reticulin, Grocott's silver stain for fungi, elastica van Giesson and Giemsa.

For localization of the p24 HIV-1 antigen in ocular tissue

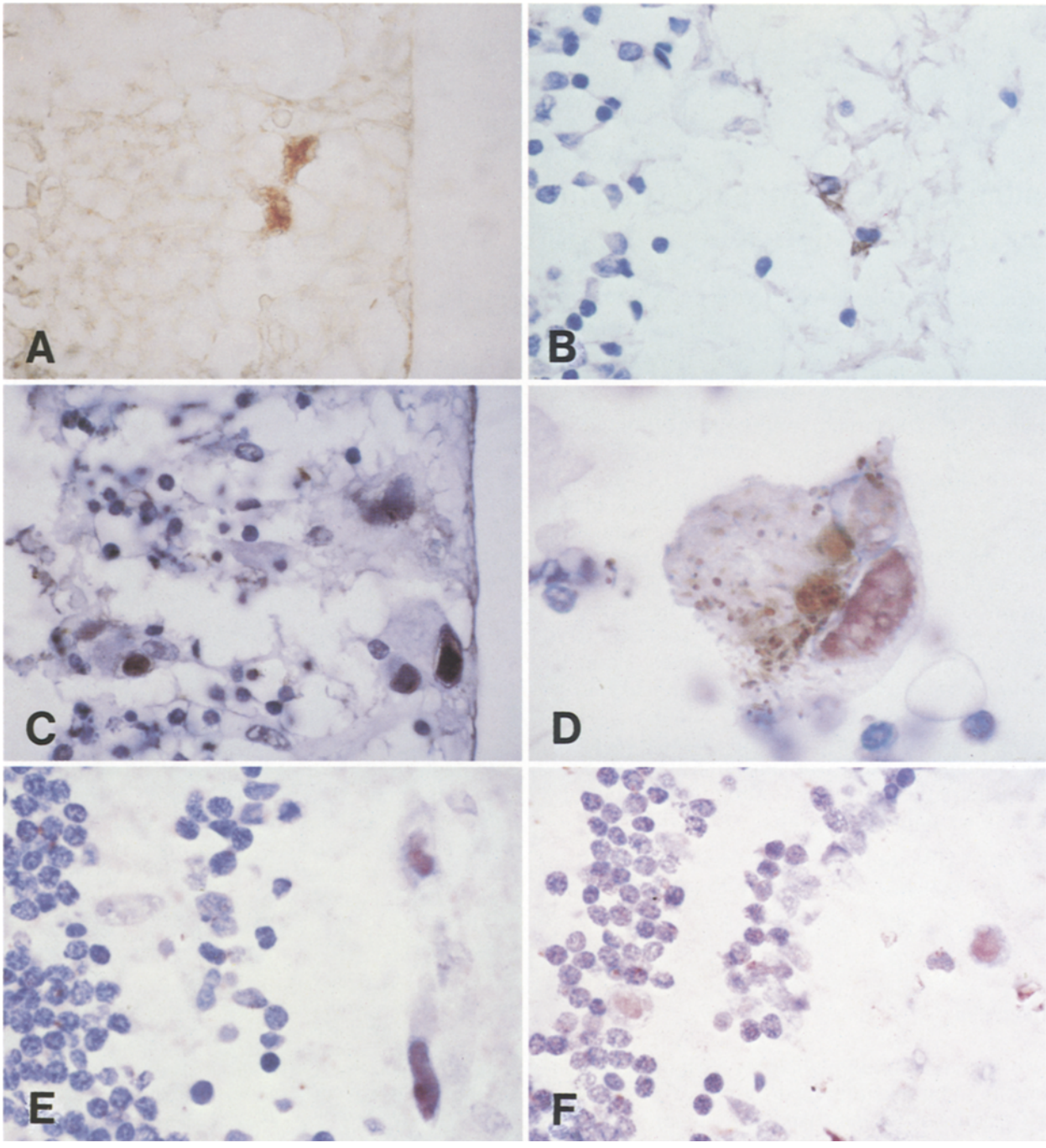

Fig. 1 A. Right eye. Scattered cells of the inner layers of the sensory retina contain the brown immunoreaction product staining p24 HIV-1 antigen (×780). Their morphology suggests a glial origin. **B** Right eye. An adjacent section stained with antibody to GFAP demonstrates typical glial cell bodies (×780). **C** Left eye. Immunohistochemistry for CMV confirms severe infection of the inner and outer nuclear and the ganglion cell layers (×450). **D** Left eye. Positive immunoperoxidase staining for CMV of a distended cell in the retinal pigment epithelium (×780). **E** Left eye. Capillary endothelial cells of the inner retina show hybridization for CMV (×780). **F** Left eye. Rare neuroretinal cells in areas with a normal lamellar architecture contain CMV nucleic acid but do not exhibit diagnostic cytomegaly (×650)

sections were incubated overnight at 4° C with a mouse monoclonal antibody to core proteins p24 of HIV-1 (kindly provided by DuPont NEN Research, Wilmington, Del) at a 1:10 to 1:100 dilution in PBS followed by incubation with horse biotinylated anti-mouse IgG and the ABC-P complex (Vector Laboratories, Burlingame, CA, USA). The peroxidase activity was developed with 3,3′diaminobenzidine (Sigma Chemical Corporation, St. Louis, MO, USA). The sections were slightly counterstained with haematoxylin and mounted in Eukitt. In addition, sections were stained using monoclonal anti-bodies to cytomegalovirus, glial fibrillary acid protein, myeloid/histiocyte antigen (Dako Diagnostika GmbH, Hamburg, FRG).

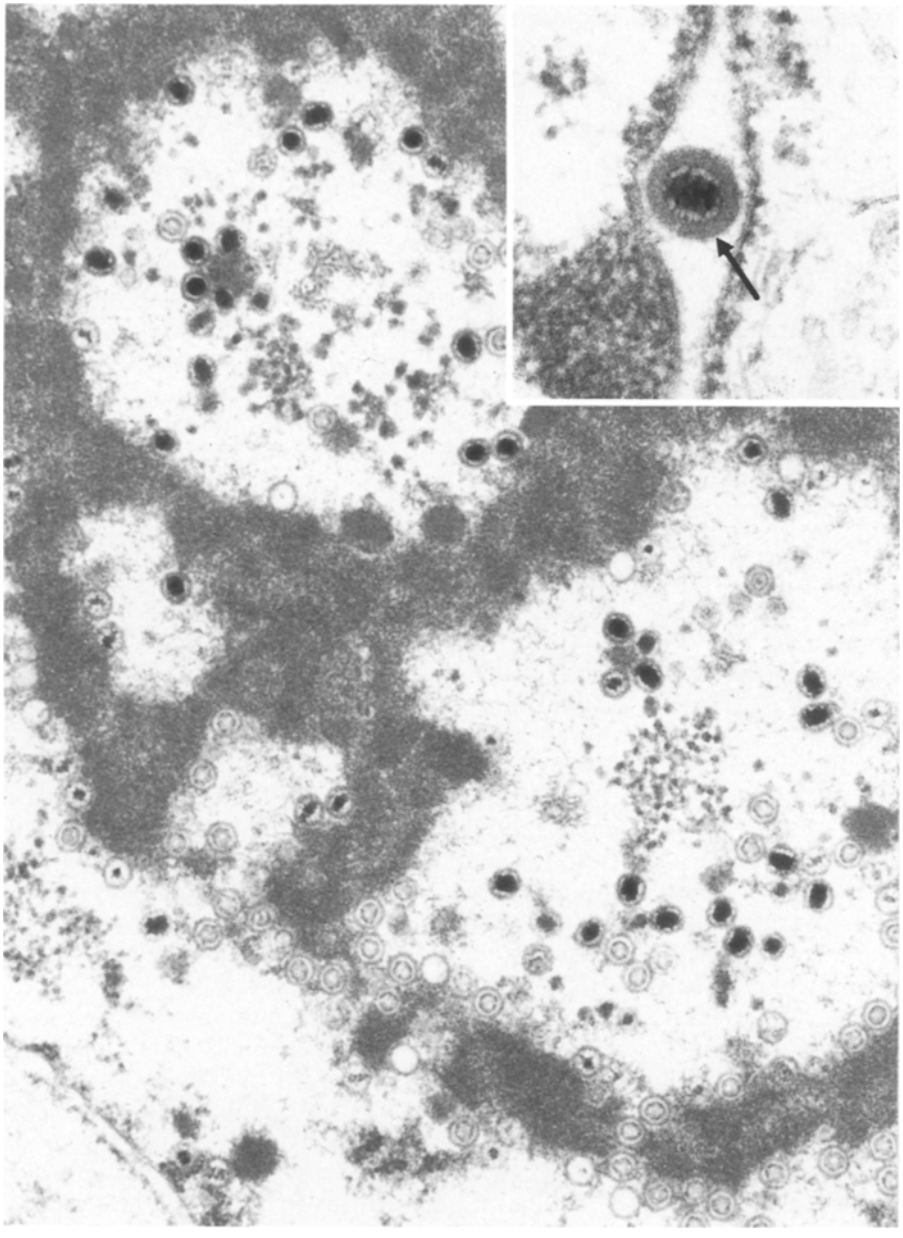

Fig. 2. Ultrastructural appearance of intranuclear viral nucleocapsids in various stages of development in the acutely inflamed neurosensory retina. Some immature virus profils lack dense bodies (×42000). *Inset.* CMV virion has budded in the perinuclear cisterna resulting in the acquisition of an envelope around the nucleocapsid and its core (*arrow*) (×69000)

Rabbit antiserum against neuron specific enolase was obtained from Incstar, Stillwater, Minnesota, USA. In order to identify Toxoplasma gondii, a rabbit polyclonal antiserum against Toxoplasma gondii was applied using a modified PAP-method as previously described (Biggemann et al. 1987).

In situ hybridization for detection of CMV nuclear acids was carried out on formalin-fixed paraffin-embedded tissue sections using commercially biotinylated CMV-DNA probes following the protocol (Enzo Biochem, New York, USA).

Portions of each retina were separated from the underlying choroid, postfixed in 2.5% cacodylate buffered glutaraldehyde followed by 1.33% osmium-tetroxide, dehydrated in graded alcohols and processed through Epon. Semithin sections were stained with toluidine blue; thin sections were double stained on copper grids with uranyl acetate and lead citrate and examined in a Zeiss EM 109.

Results

Gross pathological examination of both eyes showed no abnormalities in the anterior segment. The right retina dislayed patchy zones of thickening in the superotemporal quadrant. In the left eye, both the supero- and the inferotemporal quadrant of the retina contained withish lesions and haemorrhages. The papilla of the optic nerve was pale. The vitreous body was opaque.

Histological evaluation of the right eye showed focal oedema of the nerve fiber layer. The ganglion cells were well preserved. In both eyes, the basal

lamina of some capillaries and arterioles in the inner retinal layers were thickened. In the left eye, focal areas of necrosis were evident. Inflammatory cells were prominent around precapillary arterioles some of which were occluded. Typical cytomegalic cells were present within the zones of necrosis and in the adjacent tissue.

A small number of cells positive for p24 HIV-1 antigen was detected in the retinas of both eyes. They were characterized by strong intracytoplasmic granular staining (Fig. 1 A). Immunohistochemistry revealed the presence of p24 HIV-1 positive cells in the internal nuclear, internal plexiform and ganglion cell layers. The morphology and location of most of these cells was consistent with glia. Double-antigen immunocytochemical labeling was not performed, but when serial sections were stained with antibodies to p24 HIV-1 and GFAP, the positively staining cells were most consistent with glia (Fig. 1 B). Furthermore, some infected multinucleated giant cells were noted in the left eye adjacent to the zone of necrosis. Giant cells did not stain positively for GFAP, but they were labeled with the myeloid/histiocyte marker.

By immunohistochemical staining elements of all layers of the neurosensory retina (Fig. 1 C) and of the retinal pigment epithelium (Fig. 1 D) of the left eye contained CMV antigens. Multifocal necrotizing lesions showed numerous positively staining cytomegalic cells. In addition, CMV proteins were detected in the nuclei of scattered normal-appearing cells in areas of the retina where its architecture was well preserved. Nucleic acid hybridization with the CMV probe detected the presence of CMV nucleic acid in endothelial cells (Fig. 1 E), in cytomegalic and in a few morphologically normal cells (Fig. 1 F) of the retina. When serial sections were stained for GFAP and neuron specific enolase it was obvious that CMV infection occurred both in neurons and glial cells of the neurosensory retina. CMV was not detected in the right eye. While infection with Toxoplasma gondii was demonstrated in the brain, infection of the eyes was not observed.

In retinal specimens from the left eye electron microscopy revealed cells of all layers with intranuclear nucleocapsids in various stages of development (Fig. 2). Some capsids were empty. Rarely, budding of nucleocapsids through the nuclear membrane to the perinuclear space leading to envelopment was observed (Fig. 2, inset). Mature enveloped virions were seen freely located in the cytoplasm or in vacuoles which also contained dense bodies. By ultrastructural criteria, CMV was shown to cause productive infection within rods, bipolar ganglion, glial and endothelial cells. However, identification of the cell type was often uncertain because of extensive cytomegalic changes.

Discussion

This report has focused on ocular lesions in a patient with AIDS, who presented with cerebral toxoplasmosis, widespread CMV infection and coexistant unilateral retinitis. Clinical regression after initiating treatment with ganciclovir, an acyclic nucleoside antiviral, had been noted, but neutropenia necessiated witholding of the drug. At autopsy, severe posterior pole and peripheral retinitis was present in the left eye indicating recurrence. In the right eye, there were no findings indicating active or regressed CMV infection. Areas of oedematous nerve fiber swelling were consistent with focal retinal ischaemia.

In both eyes, retinal cells infected with HIV were evidenced by immunostaining with a monoclonal antibody to the HIV-1 p24 antigen. This observation is supported by the previous isolation of HIV from the retinas of 12 patients reported by Pomerantz et al. and Cantrill et al. (Cantrill et al. 1987; Cantrill et al. 1988; Pomerantz et al. 1987; Pomerantz et al. 1988). Moreover, HIV has been isolated from cornea, conjunctiva, aqueous humour and tears (Cantrill et al. 1987; Farrel et al. 1988; Fujikawa et al. 1985a; Fujikawa et al. 1985b; Salahuddin et al. 1986).

Although we could not establish a precise identification of the HIV-positive cells, their morphology and location mainly within the inner nuclear and plexiform layers where Müller cells are present suggest that at least neuroglial cells of the retina may be infected with HIV. Pomerantz et al. also proposed a glial cell involvement of the retina in HIV, whereas Cantrill et al. did not assess the infected cell type (Cantrill et al. 1988; Pomerantz et al. 1987). Moreover, we detected occasional positively stained multinucleated giant cells probably of monocytic origin. In contrast to what has been shown by Pomerantz et al. we were not able to detect HIV infection of retinal endothelial cells (Pomerantz et al. 1987).

Although HIV was first envisioned to have a selective tropism for T4 helper/inducer lymphocytes, it is now clear that this pathogen has a broader range of target cells (for review see Ho et al. 1987).

It is tempting to speculate that infected monocytes may migrate to the eye across the blood-retinal barrier resulting in intraocular HIV-infec-

tion. Thus, direct HIV infection of the retina may play a central role in the pathogenesis of AIDS-associated ocular lesions. However, one must take into account that only a small number of retinal cells was found to be infected by HIV. Therefore, according to what has been proposed for subacute encephalitis, AIDS-related retinal pathology may be due to a variety of mechanisms. Secretion of monokines and proteolytic enzymes within the ocular environment may have toxic effects on neuroretinal and endothelial cells.

Involvement of endothelial cells by HIV, endothelial cell damage by toxic protein released from infected cells and deposition of circulating immunocomplexes in vessel walls may result in AIDS-associated vasculopathy presenting with ischaemia.

Several lines of evidence point to a synergistic role of HIV and CMV in the pathogenesis of AIDS-associated ocular abnormalities (Freemann et al. 1984). Indeed, CMV retinitis is much more frequent in patients with AIDS than in other immunosuppressed patients (Henderly et al. 1987b).

Further investigations will contribute to the understanding of the large variety of ocular disorders in patients with AIDS and AIDS-related complex, which may result both from HIV-induced tissue damage and from the simultaneous occurrence of HIV and other pathogens in the eye.

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