A Microscopic Study of Herpes Simplex Virus Retinopathy in Mice

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ICR white mice were inoculated with herpes simplex virus (HSV) type I in the anterior chamber of one eye. Animals were killed at intervals of 2, 4, 6, 8, and 10 days and both eyes were obtained for light and electron microscopic study of retinal changes. HSV retinopathy developed in 42 (91%) of 46 inoculated eyes. Fourteen (88%) of sixteen noninoculated eyes examined after the sixth postinoculation day developed HSV retinopathy. The earliest signs of retinopathy in the inoculated eye were peripheral retinal vasculitis and inflammatory cells throughout the nerve fiber layer on day 2. No virus was found in retinal tissue until day 4, at which time disruption of outer retinal layers (outer nuclear layer and layer of rods and cones) was observed in the peripheral retina. The earliest signs of retinopathy in the noninoculated eye were isolated foci of outer retinal disruption in the posterior retina on day 6. The inflammation accompanying early retinal changes of HSV retinopathy were more severe in the inoculated eye. Electron microscopy of both eyes revealed viral particles in the inner nuclear and ganglion cell layers at the time of outer retinal disruption, but viral particles were seen only rarely in the outer retinal layers at this stage. Early disruption of normal retinal architecture may be due to infection and destruction of Muller cells. The retinopathy progressed in both eyes to total destruction of the retina by day 10. Viral infection of the retinal pigment epithelium occurred, but viral particles were seen only rarely in the underlying choroid. This model may be useful for the study of HSV retinopathy in humans. Invest Ophthalmol Vis Sci 28:1181-1190, 1987

In 1924, von Szily demonstrated that inoculation of herpes simplex virus (HSV) into a ciliary body dialysis cleft of one eye in rabbits leads to uveitis and retinopathy in the contralateral, noninoculated eye. Disease is also produced in the contralateral eye when virus is inoculated into the anterior chamber or vitreous body. A similar phenomenon can be demonstrated in mice. Originally intended for use in the study of sympathetic ophthalmia, the von Szily model and its modifications have subsequently been used by numerous investigators as a tool for the study of herpetic eye disease. It has been established that retinal disease in the contralateral eye is due to the presence of viral infection. Many studies of this model have focused on the route by which virus spreads from the inoculated to the noninoculated eye; evidence suggests a neural route of viral transmission, either through axoplasmic transport or cell-to-cell spread involving the neuroglia. Whether virus crosses to the contralateral side at the optic chiasm or elsewhere within the central nervous system remains a subject of controversy. Recent investigations have used the model to study immune responses to intraocular herpetic infections. Retinal infection results in severe, full-thickness necrosis, but little is known about the structural events occurring during retinal destruction.

In this study, retinal disease following anterior chamber inoculation of HSV in one eye was investigated further by light and electron microscopy to study the early morphologic changes that occur in the retina and the distribution of virus-infected cells. Continued investigation of this model may provide insights into pathogenesis of HSV retinopathy in humans.

Materials and Methods

Eighty-six adult ICR outbred white mice of both sexes were studied. Mice were obtained from Simon-
sen Laboratories (Gilroy, CA). Pools of RE strain HSV type I were propagated in Vero cells and stored at −70°C in 1-ml aliquots.

Prior to virus inoculation, mice were anesthetized with a 0.02 ml intramuscular injection of a 1:1 mixture of xylazine (20 mg/ml) and ketamine (100 mg/ml). A 27-gauge needle was used to make an incision at the limbus of the left eye of each mouse under microscopic visualization. A glass micropipette was placed through the limbal incision and 10³ plaque-forming units (PFU) of virus in 1 μl were introduced into the anterior chamber through a pipette attached to a Hamilton syringe. Following inoculation, the pipette was removed slowly to minimize leakage of fluid from the eye.

Animals were examined serially with a portable slit lamp for changes in corneal clarity, keratic precipitates, anterior chamber inflammation and hemorrhage, synechiae formation, and pericocular inflammation. Retinas were not examined clinically.

Animals chosen randomly from survivors were killed and their eyes were enucleated for microscopic examination on postinoculation days 2, 4, 6, 8, and 10. Selection was not based on the presence or absence of clinically-apparent ocular or neurologic disease. Both eyes from a total of 46 animals were obtained.

Following enucleation the eyes were immediately placed in 3% glutaraldehyde with 0.1% sodium cacodylate buffer at pH 7.4. Twenty-four hr later each eye was bisected. One-half were dehydrated and embedded in DuPont-Sorrall resin (Fischer Scientific, Pittsburgh, PA) for light microscopic examination. The remaining half were postfixed in 2% osmium tetroxide in Palade’s buffer, pH 7.4, for 2 hr, stained in Kellenberger’s uranyl acetate, dehydrated in ethanol, and embedded in araldite for electron microscopy.

All enucleated eyes were examined by light microscopy, in 2-μm sections stained with Giemsa stain or Lee’s stain (basic fuchsin and methylene blue). Selected ocular lesions were studied by electron microscopy, in thin sections cut on a Sorvall MT-5000 ultramicrotome (Research Manufacturing Corp., Tucson, AZ) and stained with uranyl acetate and lead citrate. Specimens were examined on a Siemens 1A electron microscope operating at 80 kv.

This study conformed to the ARVO Resolution on the Use of Animals in Research.

Results

Table 1 summarizes the relative changes in various disease characteristics observed microscopically in inoculated and noninoculated eyes through postinoculation day 8.

By day 2 all animals were found to have signs of herpetic disease in the inoculated eye by slit-lamp examination. Disease manifestations included pericellular swelling, corneal clouding and infiltration, anterior chamber reaction, and posterior synechiae. Light microscopic examination revealed numerous inflammatory cells, primarily polymorphonuclear leukocytes (PMN), and a proteinaceous exudate in the anterior chamber. PMN also were present in the iris and ciliary body and were adherent to the cornea, and there was extensive loss of corneal endothelial cells.

Twelve (92%) of thirteen inoculated eyes examined by light microscopy on day 2 had retinal disease. Inflammatory cells were present in the nerve fiber and ganglion cell layers throughout the retina (Fig. 1). Vasculitis, characterized by PMN infiltration of superficial retinal vessel walls, was present in the peripheral retina. By electron microscopy, HSV particles were identified in nuclei of iris and ciliary body cells and in remaining corneal endothelial cells of inoculated eyes. Despite the presence of inflammatory cells in the nerve fiber and ganglion cell layers, examination of several eyes failed to reveal viral particles in the retina.
There were no retinal changes or inflammatory cells in noninoculated eyes examined on day 2.

On day 4, all inoculated eyes examined had anterior segment and retinal disease. Examination revealed PMN in corneal stroma, iris, ciliary body, and anterior chamber. Numerous inflammatory cells, primarily PMN, were present in the vitreous and in the retina between the nerve fiber and outer plexiform layers; both posterior and peripheral retina were involved. Occasional PMN were located in the outer nuclear layer and photoreceptor (rod and cone) layer. Vasculitis was noted in the peripheral retina, and foci of outer nuclear layer disorganization were seen in the peripheral retina. Nuclei from this layer had moved into the photoreceptor layer, giving the appearance of photoreceptor layer “collapse.” There was nuclear enlargement with margination of chromatin in ganglion cells overlying these areas. By electron microscopy, HSV particles were identified in the peripheral retina. Virus was primarily located in the ganglion and inner nuclear layers, and was found only rarely in the outer nuclear layer and in retinal pigment epithelium.

There was no light microscopic evidence of disease in noninoculated eyes on day 4. Electron microscopic examination of several specimens failed to reveal any viral particles in retinal tissue.

By day 6 there was extensive disruption of normal retinal architecture in inoculated eyes (Fig. 2). In many specimens nuclear layers could no longer be distinguished, and nuclei were found immediately adjacent to the retinal pigment epithelium. HSV particles were identified throughout the retina (Fig. 3) and in retinal pigment epithelial cells. There was a marked inflammatory reaction in the choroid, but no viral particles were found in choroidal tissue on examination of several specimens by electron microscopy.
Ocular disease was observed first in noninoculated eyes on day 6, when two (20%) of ten animals developed evidence of anterior chamber inflammation on slit-lamp examination. Signs of anterior segment inflammation in noninoculated eyes were never as severe as those in inoculated eyes. Clinical examination was found to accurately predict the presence or absence of inflammatory cells in anterior segment structures on subsequent microscopic examination. PMN were present in iris, ciliary body, and anterior chamber, but there were no cellular changes on light microscopy to suggest viral infection of these tissues, and examination of several specimens failed to reveal the presence of viral particles. No animal had anterior segment inflammation without having retinopathy on microscopic examination. Conversely, seven (33%) of twenty-one animals subsequently found to have retinopathy in the noninoculated eye had no signs of anterior uveitis.

Seven (70%) of ten noninoculated eyes examined by light microscopy had retinal changes on day 6. Isolated foci of photoreceptor layer collapse and outer nuclear layer disruption were seen in the posterior retina (Fig. 4). The inner nuclear and ganglion...
cell layers overlying these foci were intact, although ganglion cell nuclei were enlarged with margined chromatin. A few PMN were present within the foci of outer nuclear layer disruption, and in the nerve fiber layer and vitreous overlying these foci. Viral particles were present in nuclei of the ganglion and inner nuclear layers (Figs. 5,6), and Muller cell nuclei were infected in all specimens. Enveloped and naked viral particles were present in the nerve fiber layer, but viral particles were found only rarely in the outer nuclear and photoreceptor layers in areas of photoreceptor layer collapse (Figs. 7,8).

By day 8 there was severe necrosis of the entire retina in all inoculated eyes, and retinal cells could no longer be identified. Inflammatory cells were present in the necrotic retina and in the choroid. Virus was identified only occasionally in necrotic retina by electron microscopy. In one eye viral particles were seen in a choroidal macrophage, but examination of several other specimens failed to reveal virus in choroidal tissue.

Of seven surviving animals examined on day 8, five (71%) had anterior segment disease in the noninoculated eye. All eyes examined by light microscopy had retinal disease. There was extensive disruption of the retina (Fig. 9), and nuclear layers could no longer be distinguished in many sections. Scattered PMN were present. Infiltrates were not as dense as in areas of comparable disease severity in inoculated eyes, however, and there was little inflammation in the choroid. By electron microscopy viral particles were identified throughout the retina and in the retinal pigment epithelium.

The majority of animals developed encephalitis by day 6. Few animals survived to day 10. Of nine surviving animals, six (67%) had ocular disease in the noninoculated eye. Disease was bilateral in four (44%). Retinal disease developed in the noninoculated eye, without development of retinopathy in the inoculated eye, in three (33%). In noninoculated eyes, the retina consisted of necrotic debris and scattered inflammatory cells. Retinal pigment epithelium was swollen in many areas, and Bruch's membrane remained intact. Choroidal vessels were dilated and many PMN were present in the choroid. By electron microscopy, occasional viral particles were present in the retina and retinal pigment epithelium (Fig. 10), but no virus was located in the choroid on examination of several specimens.

Discussion

The von Szily model provides a reliable method for the production of acute HSV retinopathy in animals.

Fig. 4. Light micrograph of noninoculated eye, day 6. Disruption of the outer nuclear layer with collapse of nuclei into the photoreceptor layer (white arrow) identifies an early focus of HSV retinopathy. Ganglion cells overlying the focus of collapse have margined chromatin, characteristic of HSV infection (black arrow) (X500).

The high incidence of infection and the consistency of retinal findings make it an excellent model for the study of early retinal disease.

In the study presented here, most animals developed retinopathy in both eyes. In other recent reports of this model, however, retinopathy developed only in contralateral (noninoculated) eyes. Animal species and virus strain differences have been shown to influence the severity and spectrum of herpetic disease in mice. In this study the highly susceptible ICR strain of mice may account in part for differences in retinal disease when compared with other studies. Whittum-Hudson et al hypothesize that cell-mediated immune mechanisms protect inoculated
Fig. 5. Noninoculated eye, day 6, showing viral particles within ganglion cell (GC) nuclei (open arrow) and complete virions in the nerve fiber layer (NFL) (solid arrows) (X18,800). Insert shows viral particles from the same specimen (X58,000).
eyes against retinopathy. In this study, sparing of the retina in the inoculated eye was seen only among animals surviving to day 10. In the genetically heterogeneous population of mice used, perhaps only those animals most resistant to fatal infection were able to mount an immune response that protected the retina.

This study concentrated on the early morphologic events surrounding retinal infection with HSV. In both eyes disruption of the outer nuclear layer and photoreceptor layers was the initial manifestation of retinal infection; similar changes have been reported in the retinas of rabbits given intravitreal inoculation of HSV.

While the detection of HSV in tissues by transmission electron microscopy is a relatively insensitive technique, it does provide precise information on the types of cells infected. During the early stages of infection, virus was not detected where the retina still retained its normal architecture. In areas where outer nuclear layer disruption and photoreceptor layer collapse was observed, viral particles were found in all layers of the retina. Muller cells and ganglion cells were consistently infected, but cells in the outer retinal layers were rarely infected, despite extensive early disruption of their normal architecture. The localization of virus in the inner retinal layers with sparing of
the outer layers was also observed by Pettit et al using immunofluorescence. Studies in vitro by Politi et al have shown a differential susceptibility of various retinal cells to HSV infection; photoreceptor cells appeared to be resistant to infection in that study.

It is probable that the early disruption of the outer nuclear and photoreceptor layers is caused by infection of Muller cells, the supporting neuroglia of the retina. As with other central nervous system neuroglia, the entire surface of Muller cells appears to be highly susceptible to HSV attachment and penetration. Neurons are susceptible only at the synaptic endings, but are resistant on the perikaryon. It is hypothesized that in this animal model, virus reaches the retina via retrograde transport in axons of the optic nerve to produce infection of ganglion cell nuclei. Virus released from ganglion cells is then taken up by Muller cells, the destruction of which leads to the early disruption of the outer retinal layers. Virus can then spread throughout the retina to infect both synaptic nerve endings and other contiguous Muller cells.

Muller cell processes also form the inner and outer limiting membranes of the retina. The internal limiting membrane would be the first retinal tissue encountered by virus diffusing posteriorly from the anterior chamber of inoculated eyes, thus explaining early disruption of the peripheral retina.

Inoculated eyes consistently developed a greater inflammatory response than noninoculated eyes. In inoculated eyes, PMN were seen throughout the inner layers of the retina preceding herpetic infection of retinal cells. The lack of viral particles and the presence of peripheral retinal vasculitis suggests that the early inflammatory response may have been produced by local spread of immune mediators from the inflamed anterior segment. In contrast, noninoculated eyes developed a milder inflammatory reaction only in areas of retinal disruption; nevertheless, retinal necrosis followed a similar course in both eyes. It
is therefore likely that the inflammatory response did not play a major role in retinal destruction in the noninoculated eye.

The von Szily model may prove to be useful for the study of human HSV retinopathy. Because it is free of injection artifact, and because virus reaches the eye by an endogenous route, the noninoculated eye may be a useful model of HSV infection of the retina in humans, which also results in severe retinal necrosis.\textsuperscript{15-19} As in the mouse model, HSV has been found more frequently in the inner layers of the retina and in the retinal pigment epithelium of these human cases.\textsuperscript{15,16} Cibis and Flynn found margined chromatin, inclusion bodies and virus particles primarily in the ganglion cell and inner nuclear layers of an 18-month-old infant with HSV encephalitis.\textsuperscript{17} In adult patients with HSV retinopathy, Minckler et al observed that maximum necrosis occurred in the inner retina,\textsuperscript{18} and Johnson and Wisotzkey found Cowdry type A eosinophilic intranuclear inclusions in ganglion cells, but few inclusions in outer retinal layers.\textsuperscript{19} These findings are consistent with the observed distribution of HSV in this animal model. In contrast to very early degeneration of photoreceptors in this model, however, Minckler et al found areas of photoreceptor layer preservation in their patient.\textsuperscript{18} Specimens of human HSV retinopathy have not been examined during very early stages of disease; most human tissue becomes available only after wide-

**Fig. 9.** Light micrograph of noninoculated eye, day 8, showing extensive disruption of retinal architecture. There is widespread collapse of the outer nuclear layer into the photoreceptor layer. Inner limiting membrane and nerve fiber layer cannot be identified. There is little choroidal infiltration (x500).

**Fig. 10.** Noninoculated eye, day 10. Intranuclear particles are seen in a retinal pigment epithelium (PE) cell. Bruch's membrane is intact (B). No virus was seen on examination of the choroid (C) (x13,700).
spread destruction of the retina has occurred, which emphasizes the need for an appropriate animal model for research.

Key words: herpes simplex virus, mice, Muller cell, retinopathy, von Szily model

References