Ocular Lesions in Mice Following Intracerebral Injection of Herpes Simplex Virus Type I

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A clinical isolate of type I Herpes virus was injected intracerebrally in 4-week-old Balb/C mice. Bilateral ocular disease was observed initially clinically as a leukocoria and an anterior uveitis on the 7th to 11th postinjection days. By day 21 an organized vascularized retrolental membrane had formed with resolution of active inflammation and secondary cataract formation. Light microscopy revealed the process to involve a necrotizing retinitis with associated optic nerve demyelination. Electron microscopy and tissue culture demonstrated the virus in involved tissues. Invest Ophthalmol Vis Sci 24:1070-1078, 1983

Herpes simplex virus (HSV) is a common cause of adult encephalitis in persons. HSV includes two subtypes that have distinctly characteristic clinical and pathologic manifestations. HSV Type I (HSV-I) produces lesions in nongenital areas including the cornea, skin above the waist, oral mucous membranes, and the central nervous system; while rarely isolated from cerebrospinal fluid, the virus can be cultured from infected brain tissue. HSV-I retinitis has been demonstrated in individuals with encephalitis. Type II (HSV-II) is transmitted as a veneral infection, producing lesions of the genitalia and skin below the waist. In newborns, it may affect any organ; transplacental infection has been postulated to explain a variety of congenital defects, including cataracts and chorioretinitis.

In a case of newborn HSV chorioretinitis, subtype not specified, retinal gliosis and retinal pigment epithelial (RPE) hyperplasia were attributed to "immunologic processes" rather than direct invasion by the virus, and no viral inclusions were found. HSV (subtype not specified) was cultured from the anterior chamber of a neonate with bilateral panuveitis; clinical ocular lesions included visual impairment and retinal exudates and hemorrhages.

Animal models of HSV uveitis and associated pathology were first described in 1924 when von Szily, in an attempt to produce experimental sympathetic ophthalmia, inoculated HSV into a dialysis cleft between the ciliary body and the sclera in one eye of rabbits; uveitis developed in all the inoculated eyes and in 15% of fellow eyes 10-14 days later. Gifford and Lucie repeated these experiments with similar results; histopathologic evidence from both studies suggested that the virus reached the uninoculated eye via the optic nerves. Goodpasture studied similar models to support the concept of axis cylinder propagation, while Marinesco and Dragenesco concluded from their studies that the virus spread along the "lymphatic channels" of the nerves. Kimura injected PH strain of HSV unilaterally into the anterior chamber of rabbits; 74% developed a severe uveitis in the fellow eye within 7 to 12 days. HSV was isolated from the optic nerve and retina of the uninoculated eye. Pettit and his colleagues used a similar model and fluorescein-labeled antibodies to suggest transmission to the fellow eye occurred hematogenously rather than directly through neural tissue. Kristensson and associates injected rabbit virulent with G strain HSV-II; virus particles were found in the ipsilateral ganglion cells and contralateral superior colliculus and lateral geniculate body. Later work demonstrated arrest of myelination and demyelination of retinal ganglion cell axons in young and adult rabbits, respectively.

Percy et al induced experimental type II herpes ophthalmitis in the newborn rat; following intracerebral injection, keratitis, iritis, and retinitis were demonstrated. Brick et al used newborn rabbits to...
demonstrate systemic HSV-II infection following subcutaneous inoculation; chorioretinitis was observed in 40% of the animals. This study was undertaken to determine if similar disease might be experimentally induced with HSV-I in 4-week-old immunocompetent mice.

Materials and Methods

One hundred 4-week-old female Balb/C mice were inoculated with four plaque-forming units (PFU) of a HSV-I clinical isolate obtained from a perianal lesion of an immunodeficient child. The stock titer of the virus pool was \(7.95 \times 10^6\) PFU/ml. Technique involved light ether anesthesia and injection of 0.05 ml of virus suspension into the right cerebral hemisphere with a 27-gauge needle and tuberculin syringe. Concomitant titration used intracerebral inoculation of groups of four mice with one of five serial tenfold dilutions of the virus pool \((10^{-3} \text{ to } 10^{-8})\), observation for 19 days, and calculation using the modified Karber method; results indicated that 0.05 ml of virus suspension represented 0.5 LD\(_{50}\). Ten control animals were injected with sterile phosphate buffered saline and along with ten uninjected mice were subjected to described examination techniques.

Mice were subjected to clinical ocular examination just prior to inoculation and on days 2, 4, 7, 11, 14, 21, 28, and 84 postinoculation; technique involved dilation of the pupil with 1.0% tropicamide (Mydriacyl® Alcon Laboratories, Ft. Worth, TX), manual restraint, and slit-lamp biomicroscopy of the adnexa, anterior segment, and anterior vitreous and direct and indirect ophthalmoscopy of the posterior segment.

Four animals were chosen randomly from healthy and sick survivors and killed on days 1, 2, 4, 6, 9, 11, 14, 21, and 29 postinoculation; five animals were killed on day 84. Following deep ether anesthesia and cervical dislocation, one eye of each animal was removed for virus culture. Three fellow eyes were fixed tiny pupil, engorgement of iris vasculature, and anterior cortical cataracts appeared as early as day 11 and progressed slowly (Fig. 1C).

Between days 14 and 28, intensity of the anterior segment inflammation subsided, and the necrotic retina became less edematous and appeared to organize into glial membranes (Fig. 1B). Anterior and posterior cortical cataracts appeared as early as day 11 and progressed slowly (Fig. 1C).

Culture Results

Positive cultures were obtained from brain tissue of one mouse out of four on days 4 and 6: two of four on day 9; and one of four on day 14. Positive cultures were obtained from the optic nerves at the level of the chiasm from one of four animals on days 9, 11, and 14. Positive cultures were obtained from the trigeminal ganglia from one of four mice on day 14. Ocular tissues were positive in one of four eyes on days 9 and 11 and in two of four eyes on day 14. Positive cultures were not obtained from any tissues after day 14.

Histology

Light microscopy of ocular tissues allowed clinicopathologic correlations and elucidation of disease pathogenesis. Earliest changes were observed on day 7 and included retinal edema that affected primarily...
Fig. 1. Clinical course of representative ocular lesions following intracerebral injection of HSV-1 included (1) anterior uveitis, manifested by flare and cells, engorgement of iridal vasculature, pupillary resistance to pharmacologic dilation, and hyphema, and (2) leukoria due to retinal edema on day 7 (A); resolution of inflammation with formation of a vascularized, focally calcified retrolental membrane by day 12 (B); and formation of secondary cataract by day 28 (C). All photographs approximately 4X.
Fig. 2. Histologic features during the early course of the disease (7–10 days) include a predominately mononuclear inflammatory cell of the iris (i) and anterior chamber (c-cornea) (A); swelling, margination of chromatin, and eosinophilic inclusions (arrow) of ganglion cell nuclei (B); and similar inclusions (arrows) in bipolar cell nuclei (C) (hematoxylin-eosin; original magnification A, 500×; B, C, 1250×).
the area adjacent to the optic disc and the inner retinal layers. Eosinophilic intranuclear inclusions with chromatin margination were present in ganglion cells. A mild panuveitis with a mixed neutrophilic and lymphocytic component was present, and cells and proteinaceous exudate were observed in the vitreous and anterior and posterior chambers (Figs. 2A–C).

By day 14, the entire retina was necrotic and disorganized. Inclusions were present in degenerating bipolar and RPE cells. The choroidal infiltrate had
Fig. 4. By day 21, the choroidal infiltrate had resolved and the necrotic retina had organized into a calcified gliotic membrane that filled the vitreous cavity; multinucleated giant cells are seen within the membrane. Posterior migration of lens epithelium and liquefactive pools of cortical protein characterized secondary lens changes (1) (hematoxylin-eosin; original magnification 125X).

became intense, consisting of primarily lymphocytes and plasma cells with occasional histiocytes (Figs. 3A, B). The anterior uveitis persisted, and neutrophils were observed within the corneal stroma. Gliosis and demyelination were observed in the optic nerve.

By day 21, the necrotic retina had been replaced by a gliotic membrane with areas of calcification and numerous multinucleated macrophages. No identifiable retinal cells, including RPE, was present. The mononuclear choroidal infiltrate was less intense. Early cataractous change included posterior migration of lens epithelium and degeneration of posterior lens fibers (Fig. 4). Resolving anterior uveitis was hallmark by occasional mononuclear cells and posterior synechiae. After day 21, findings included continued resolution of active inflammation, organization and calcification of gliotic retina and progression of cataracts.

Electron Microscopy

The ultrastructural morphology of the retina and optic chiasm in all mice killed from days 1 through 5 postinoculation were unremarkable. Viral particles were observed in optic nerve glial cells and ganglion cell nuclei on day 6 (Figs. 5, 6A). On day 11, virus particles were seen in the inner and outer plexiform layers with associated retinal necrosis. By day 14, a degenerative demyelinating condition was observed in the optic nerve characterized by plasma cells, occasional cosinophils, and numerous lipid-laden phagocytic cells containing degenerative myelin fragments (gitter cells). Glial cells were decreased in number, and a few degenerated oligodendroglia and astrocytes contained herpes simplex nucleocapsids (Fig. 6B). At this time the retina exhibited severe pathologic alterations, with all layers involved in a necrotizing process. Necrotic cells contained herpes simplex virus with and without envelopes. Partially calcified cellular debris, and a few neutrophils and cosinophils were identified within the disorganized retina. Viral particles were observed in degenerating RPE cells. The choroid was markedly thickened by an infiltration of lymphocytes, monocytes, cosinophils, plasma cells, and neutrophils.

Discussion

Only a few viruses other than herpes have been shown to cause intraocular disease. These include human infections with rubella virus, cytomegalovirus, and the virus of subacute sclerosing panencephalitis. Bovine vial diarrhea virus causes retinopathy in the fetal calf, and SV-40 virus, reovirus type 3 and lymphocytic choriomeningitis virus produce retinopathy in newborn rats. Blue tongue virus infection of the pregnant ewe causes retinal dysplasia in the newborn lamb. Canine distemper virus may result in chorioretinal lesions. Scrapie agent will cause retinopathy in hamsters and sheep. Pathogenesis of viral retinopathies may involve direct virus invasion of retinal cells, immunologic mechanisms, or a combination of both processes.

In this study, bilateral ocular disease caused by an HSV-I clinical isolate was observed in 6 of 64 survivors by the eleventh postinoculation day. Morphologic observations suggested a primary necrotizing retinitis by centrifugal pericentral rather than hematogenous spread due to (1) a central to peripheral and outer to inner retinal gradient of involvement in the early stages of the disease process; (2) identification...
Fig. 5. Electron microscopy of retina demonstrated viral particles (arrows) within ganglion cell nuclei on day 6 (A) and within degenerating bipolar cells (B) and retinal pigment epithelial cells (C) by days 9–14.
of virus particles within the optic nerve and retinal ganglion cells concurrently; and (3) infiltration of the choroid late in the disease subsequent to extensive retinal involvement. However, this study did not specifically address this question and the possibility of hematogenous spread via the retinal vasculature in addition to or rather than centrifugal spread cannot be excluded. All retinal cell layers, including the RPE, were eventually involved and infected; Font described similar panretinal involvement with the virus of subacute sclerosing panencephalitis.36

Uveal involvement appeared to be secondary; viral particles or inclusions were not seen in choroid, ciliary body, or iris. Witner and Iwamoto described infection of iris stromal cells, muscle cells, melanocytes, and pericytes in a patient with herpetic iritis37; in the mouse model described, no such lesions were observed. Likewise, we were unable to demonstrate viral infection of cornea and iris, as did Percy et al19 in experimental HSV-II ophthalmitis in rats.

The disease resolves with formation of a calcified glial membrane; the calcium may be released from degenerating photoreceptors. Cataracts appear to be secondary to the inflammation rather than to direct virus invasion of lens fibers or epithelium.

Morphologically, the juvenile mouse herpetic retinitis model described appears similar to that described in neonatal rats.19 This study complements previously published work by introducing another species, demonstrating that the condition can be induced in immunocompetent animals,38 and incriminating HSV-I as a intraocular pathogen.
Key words: herpes simplex virus type I, mouse, retinitis, optic nerve demyelination

References


