Ocular Disease Induced in Mice by Anterior Chamber Inoculation of Herpes Simplex Virus

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Herpes simplex virus type 1 (HSV-1) inoculated directly into the anterior chamber of the mouse eye induced an acute inflammatory process in both the injected eye and its uninjected (contralateral) counterpart. In the former, a rapid intense inflammatory reaction developed in the anterior segment (cornea, anterior chamber) and anterior portion of the uveal tract (iris and ciliary body). The retina of the injected eye was spared. In contrast, in the uninjected eyes, a delayed massive destructive reaction also developed, but was limited almost exclusively to the posterior segment (vitreous, retina and choroid); retinas of the uninjected eyes were destroyed completely. When HSV-1 was inoculated bilaterally into both anterior chambers, destructive inflammatory responses developed in both corneas and anterior segments, but the retinas were spared bilaterally. These results indicate that (1) a unique and interesting pattern of bilateral ocular disease occurs after uniocular anterior chamber injection of HSV-1 in mice; (2) the distribution of the destructive lesions differs between the injected eye and its uninjected counterpart; and (3) local factors, perhaps produced within the eye itself, modify the progression of the virus-induced reaction within the globe. Invest Ophthalmol Vis Sci 25:1065-1073, 1984

Herpes simplex virus type 1 (HSV-1) infections are a leading cause of corneal disease and blindness. Unlike HSV-1 infections at other tissue sites, which are non-progressive and characterized chiefly by recurrent, self-limited epithelial lesions, HSV-1-induced ocular disease may involve the cornea (epithelium, stroma, endothelium), the anterior uveal tract (iris, ciliary body), and/or the posterior segment (vitreous, retina, choroid).1 The pathogenic mechanisms responsible for the ocular disease resulting from HSV-1 infection are not understood completely. It is not clear to what extent direct virus-induced cytopathology contributes to the ocular lesion, as opposed to an immune-based pathogenesis in which immune effectors attack and destroy virus-infected tissues.2 It is clear that individuals infected intraocularly with HSV-1 make immune responses to viral antigens.3-7 However, despite extensive study of both humans with HSV-1 ocular disease and animal models developed in rabbits6-11 and mice,12-13 the relative participation of humoral and cell-mediated responses to the virus in the pathogenesis of herpetic ocular disease is unresolved.14-22 One recent study compared the extent of herpetic keratitis in normal and athymic (nu/nu) BALB/c mice and revealed a milder disease in the latter animals,13 suggesting that immune-mediated processes may contribute to the severity of the corneal disease.

We have been studying the deviant immune responses mice make to alloantigens introduced into the anterior chamber of the eye.23-26 The availability of numerous inbred mouse strains plus many useful immunologic reagents has made this approach advantageous in studying the relationship of immunologic function to the pathogenesis of herpetic ocular disease. Herpes simplex virus inoculated intracamerally, in a manner similar to alloantigenic tumor cells23-26 and haptenated lymphoid cells,27-28 induces anterior chamber-associated immune deviation (ACAID); systemic T-cell-mediated responses (delayed hypersensitivity) to HSV are depressed, while humoral anti-HSV immunity remains intact or is enhanced.29 When eyes from mice infected intracamerally with HSV-1 were examined by slit-lamp biomicroscopy, changes were observed that were both striking and unusual. In this report, we describe the clinical and pathologic findings on eyes of mice infected intracamerally with HSV-1.

1065
Materials and Methods

Mice

Young adult female BALB/c mice were obtained from Cumberland View Farms (Clinton, TN) and maintained in our animal colony. In utilizing animals in this study, we provided adequately for their care and humane treatment, and adhered to the ARVO Resolution on the Use of Animals in Research.

Virus

HSV-1 (KOS strain) stock was kindly provided by Dr. Lewis Pizer (University of Colorado Medical School; Denver, CO). Virus was propagated and titrated by the method of Schrier et al.30 in rabbit skin cells, which were received also from Dr. Pizer.

Inoculation of HSV-1

Inoculation into the anterior chamber has been described previously.24 Briefly, BALB/c mice were anesthetized by intramuscular injection of 0.66 mg of ketamine hydrochloride (Vetalar; Parke, Davis & Co.; Detroit, MI). While viewing the proposed eye under a dissecting microscope, a 30-g needle was used to puncture the cornea midway between the limbus and central cornea. After leaking aqueous was blotted, 2 nl of virus suspension in Dulbecco’s Minimal Essential Medium (MEM) were injected with a glass micropipet connected to a Hamilton syringe into the anterior chamber. The virus injection was given through the hole made with the 30-g needle. In some experiments, 2 nl of MEM were inoculated into the contralateral eyes to exclude injection trauma as a cause of inflammatory reaction. Additional control mice received the same dose of HSV-1 either intravenously or subcutaneously. In both of these latter groups, eyes remained normal through 21 days (data not shown).

Clinical Evaluation

At varying timepoints after intraocular inoculations, mice were examined by slit-lamp biomicroscopy. The anterior segments were evaluated for the presence of inflammation on a scale of 0 (normal) to 4+. The posterior segments were not visualized. At the time of killing, eyes were enucleated and preserved in buffered formalin until processing for light microscopy.

Histopathology

Because corneal involvement obscured changes in the posterior segment of infected eyes, it was necessary to accompany this study with a histopathologic analysis of HSV-1 infected ocular tissues in order to describe the changes not observable by slit-lamp microscopy.

Fixed eyes were embedded in paraffin, serially sectioned at 5 μm, and stained with hematoxylin and eosin. In all cases, histology confirmed slit-lamp diagnosis, so that the sign diagnosed by both methods at each time point reflected the larger number (n) of eyes examined by slit lamp.

Virus Titration

In selected experiments, eyes were enucleated and homogenized for viral titration on triplicate rabbit skin cell monolayers. Pooled injected or uninjected eyes (2-4/group) were minced finely in 0.5 ml medium (MEM). Serial 10-fold dilutions were prepared and 0.1 ml of suspension was applied to each 60-mm petri dish containing approximately a 70% monolayer of rabbit skin cells. After a 2-hr incubation at 37°C in a CO2 atmosphere, 3 ml of a 1.2% agarose solution in MEM containing penicillin (100 IU), streptomycin (100 μg/ml) and 10% newborn calf serum were overlaid onto the monolayer. Plates were incubated for 3–5 days and observed for cytopathic effects (CPE). After the indicator cells developed CPE, plates were fixed with buffered formaldehyde and stained with 0.1% crystal violet for 15–20 min, followed by rinsing with tap water. Titers were determined by the method of Reed and Muench.31

Results

Inoculation of HSV-1 (KOS) into the anterior chamber of BALB/c mouse eyes produces an intense inflammatory reaction at the inoculation site. The intensity and tempo of the inflammatory reaction are dose-dependent over a wide range: 2 × 102 to 2 × 105 pfu HSV-1. For the studies to be described, we chose the dose of 2 × 104 pfu for the following reasons: (1) this dose of virus induced anterior chamber-associated immune deviation (ACAID), ie, recipient mice produce high titers of circulating anti-HSV-1 antibody, but fail to develop T-cell immunity as measured by the capacity to express HSV-1-specific delayed type hypersensitivity (DTH)30; (2) when inoculated subcutaneously, this dose of virus regularly induces vigorous DTH to HSV-1 as well as humoral anti-HSV-1 responses; and (3) lid vesicles were observed uncommonly after AC inoculation of 2 × 104 pfu HSV, whereas at higher doses lid lesions regularly occurred, raising the possibility that auto-inoculation of virus by infected mice would unduly confound the analysis. Within this dose range, the only visible manifestation of disease in AC-inoculated mice was in the injected eye or its cohort. None of the mice died or developed signs of disease at other sites. However, histologic examination and virus isolation demonstrated the development of focal necrotizing encephalitis. The presence of encephalitis is consistent...
Table 1. Time course of ocular changes following intracameral inoculation of HSV-1

<table>
<thead>
<tr>
<th>Site</th>
<th>Sign</th>
<th>Injected eye</th>
<th>Uninjected eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lids</td>
<td>Lesions</td>
<td>5 (2/5)†</td>
<td>10 (2/8)</td>
</tr>
<tr>
<td>Cornea</td>
<td>Edema</td>
<td>1 (13/13)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>Neovascularization</td>
<td>1 (7/13)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>Infiltration</td>
<td>1 (1/13)</td>
<td>(-)</td>
</tr>
<tr>
<td>Anterior chamber</td>
<td>Loss of form</td>
<td>3 (11/15)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>(depth)</td>
<td>11 (4/7)</td>
<td>(-)</td>
</tr>
<tr>
<td>Iris</td>
<td>Cell and flare</td>
<td>1 (3/3)</td>
<td>7 (9/12)</td>
</tr>
<tr>
<td></td>
<td>Infiltration</td>
<td>1 (13/13)</td>
<td>7 (9/12)</td>
</tr>
<tr>
<td></td>
<td>Atrophy</td>
<td>1 (6/13)</td>
<td>3 (3/17)</td>
</tr>
<tr>
<td></td>
<td>Pupillary</td>
<td>(-)</td>
<td>7 (5/6)</td>
</tr>
<tr>
<td>Lens</td>
<td>Cataract</td>
<td>1 (3/3)</td>
<td>10 (3/3)</td>
</tr>
<tr>
<td>Vitreous</td>
<td>Infiltration†</td>
<td>3 (3/3)</td>
<td>3 (3/3)</td>
</tr>
<tr>
<td>Retina</td>
<td>Infiltration‡</td>
<td>1 (3/3)</td>
<td>3 (3/3)</td>
</tr>
<tr>
<td></td>
<td>Necrosis‡</td>
<td>(-)</td>
<td>14 (3/3)</td>
</tr>
<tr>
<td>Choroid‡</td>
<td>Infiltration**</td>
<td>(-)</td>
<td>14-21 (3/3)</td>
</tr>
</tbody>
</table>

* Observations were made with a slit-lamp biomicroscope or by histologic examination of H- and E-stained sections of eyes removed on days 1, 3, 5, 7, 10, 14, and 21 postinoculation of 2.4 × 10^6 pfu of HSV-1 into one eye of each BALB/c mouse.
† Day postinoculation (number of positive eyes/total eyes (n) examined): day 1, n = 13; day 3, n = 17; day 5, n = 5; day 7, n = 12; day 10, n = 8; day 14, n = 6, day 21, n = 3. At timepoints earlier than 21 days when histological evaluation only was performed, n = 3. (-) indicates not effected.
‡ Histologic evaluation only except time of onset of cataract in injected eyes.
§ Injected eye, anterior infiltrate; uninjected eye, posterior infiltrate.
¶ Injected eye, minimal infiltrate limited to ganglion cell layer (GCL); uninjected eye: a pinpoint focus of proliferation or infiltration initially limited to GCL and finally involving entire retina.
|| Early necrosis extended through all retinal layers but was demarcated by normal retinal architecture; within 4-5 days, entire retina was necrotic.
** Underlying necrotic retina only.

With findings of Price et al., who studied spread of HSV to the central nervous system following anterior chamber inoculation of HSV-1. The KOS strain was chosen because it is relatively nonpathogenic in mice by a variety of routes.

On day 0, a group of 23 BALB/c mice was inoculated with live HSV-1 (2 × 10^6 pfu) into one anterior chamber per mouse. At several points thereafter, both injected and uninjected contralateral eyes were examined by slit-lamp biomicroscopy. At each time point, two or three mice were killed and their eyes removed for tissue sectioning and histopathologic study. The results of slit-lamp and histologic observations are summarized in Table 1. Because biomicroscopy of the posterior segment of mouse eyes is exceedingly difficult, examination of this portion of the eye was conducted exclusively from histologic sections (Table 1, footnote).

The major findings in the anterior segment were that (1) eyes injected with virus developed a severe inflammatory reaction in the cornea and anterior uveal tract (keratouveitis) within 1–3 days; (2) cataracts developed and matured by day 21; (3) contralateral eyes that had not received virus exhibited a moderate uveitis after a lag period of 5–7 days, but corneas and anterior chambers of these uninjected eyes remained free of inflammation; and (4) cataracts were observed to develop in these eyes by day 21. Thus, HSV-1 inoculated into the anterior chamber of mouse eyes produces an acute anterior segment inflammation. Involvement of the contralateral (uninjected) eye was observed, but did not involve the cornea, suggesting that pathologic changes in the noninjected eye were not the result of auto-inoculation from the injected eye.

It was this observation that led us to conduct a full-scale histopathologic analysis of both injected and uninjected eyes in order to gain insight into the nature of the disease in the contralateral eye. Details of both the biomicroscopic and histopathologic analysis are described below.

**Cornea**

Unilateral intracameral inoculation of HSV-1 produced an intense inflammatory reaction of the anterior segment of virus-injected BALB/c eyes which evolved during the period of 1–7 days postinoculation (PI): corneas rapidly became edematous (1–3 days PI); infiltration (one of three eyes) and neovascularization (seven of thirteen eyes) were observed by day 1 (Fig. 1A). The central stromal infiltrate peaked by day 7 and resolved completely by day 21. Extensive neovascularization reached maximum intensity by day 14 and persisted until killing (day 21). Histologic examination revealed an acute inflammatory infiltrate surrounding the injection site. The extensive loss of corneal clarity observed by slit lamp corresponded to edema and disruption of the lamellar array of the corneal stroma. By day 5, the corneal endothelium had been destroyed.
Fig. 1. Histopathology of injected eyes. Photomicrograph of sections from injected eyes 7 (A, D, G), 10 (B, E, H), and 14 (C, F, I) days after unilateral inoculation of $4 \times 10^4$ pfu of live HSV-1 (KOS) into the anterior chamber. Ocular structures shown are cornea and anterior chamber (A-C), ciliary body (D-F), and retina (G-I). The same magnification was used in all panels. Bar equals 40 μm. Reproduced with permission from the publisher. Whittum JA, McCulley JP, Niederkorn JY, and Streilein JW: Unilateral inoculation of herpes virus into the anterior chamber produces a bilateral uveitis with a pathogenesis potentially related to anterior chamber-associated immune deviation (ACAID). In Proceedings of the Third International Symposium on Immunology and Immunopathology of the Eye, Chandler JW and O'Connor GR, editors. New York, Masson, 1984 in press.

In contrast, contralateral (control) corneas remained normal throughout the examination period (Figs. 2A–C). One-half of the control eyes were injected with diluent alone in order to control for nonspecific effects secondary to the injection itself. Only transient corneal edema with a mild inflammatory response developed and resolved by day 3 in all cases. The complete lack of corneal pathology in control eyes argues against an external spread of virus from the injected to the uninjected eye.

Anterior Chamber

The anterior chambers of injected eyes contained an increasingly severe cell and flare reaction visible by 1 day after virus inoculation (Fig. 1A). Over 21 days, the chambers became progressively more shallow (loss of form or depth) and were occupied by fibrovascular tissue between days 14 and 21 PI. The anterior chambers of contralateral eyes developed a mild inflammatory exudate by day 7. This exudate persisted.
through day 21, but the chamber retained its normal depth throughout this period (Figs. 2A–C).

**Anterior Uveal Tract (Iris and Ciliary Body)**

In the injected eye, uveitis was observed early (by day 1); iris infiltration (13 of 13 eyes) and loss of iris integrity (6 of 13 eyes) were both evident at this time. Iris atrophy (loss of iris stroma and vessels) occurred in 6 of 15 eyes by day 3. Slit-lamp visualization of the anterior uveal tract of the injected eye was not possible between days 3 and 14 PI because of extensive corneal inflammation. Histologic examination revealed that loss of integrity of both the iris and ciliary body was
maximal at day 7 PI (Fig. 1D). No particular effects upon the anterior choroid were observed throughout the examination period.

Examination of the anterior uveal tract of contralateral (nonvirus injected) eyes revealed a minimal to moderate inflammatory infiltrate in the iris, which developed in a delayed fashion between days 7 and 10 PI. This infiltration extended to the ciliary body (Figs. 2D–F). By day 10, disruption of the posterior layer of the iris had occurred. We observed regularly that inflammation in contralateral eyes was less severe and delayed by approximately 1 week compared with pathologic changes in virus-injected eyes.

Lens

Cataracts developed in all eyes. Corneal opacity prevented visualization of mature cataracts in injected eyes by slit-lamp microscopy. However, histologic examination demonstrated that most lenses were swollen by day 5, infiltrated by day 10, and the lens epithelium had begun to degenerate by day 14. By day 21 PI, the injected eyes were shrunken (phthisic). Cataract development in un.injected eyes was delayed by about 2 weeks; capsular fibrosis and shrinkage of the lens had begun to occur by day 14. By day 21, migration of lens epithelium and further fibrosis was evident.

Posterior Segment (Vitreous, Retina, and Choroid)

The contrast between the pathologic processes in the posterior segments of the injected and contralateral control eyes was most striking. Although the anterior vitreous of injected eyes was infiltrated with inflammatory cells (from day 3 on), retinal architecture remained normal in these eyes throughout the experimental period. The only exception was a minimal inflammatory infiltrate in the ganglion cell layer (GCL) of the retina (Figs. 1G–I). This infiltrate persisted from day 1 to the conclusion of the experiment.

Histologic examination of serial H and E sections of contralateral uninoculated eyes revealed several pathologic changes in the posterior segment. A mild inflammatory infiltrate was present in the anterior vitreous by day 7, but this infiltrate did not appear to be contiguous with other small areas of inflammation that were located adjacent to the retina. The posterior infiltrate that developed subsequently appeared to correlate with extensive retinal changes described below.

The retina remained normal until day 7 PI, at which time small foci of inflammatory cells appeared around one or two small vessels in the ganglion cell layer (Fig. 2G). These foci were limited generally to one lateral aspect of the GCL per eye, midway between the optic nerve and the anterior edge of the retina ( ora serrata). The remainder of the GCL appeared normal at this time as did the outer layers of the retina and underlying choroid. As shown in Figure 2H, by day 10 a large necrotic area containing a mixed inflammatory infiltrate extended posteriorly through the entire retina. The choroid underlying the necrotic area also was infiltrated. Inflammatory cells could occasionally be seen around the optic nerve at this time.

By day 14 PI, the retina was completely necrotic with inflammatory cells, debris, and numerous plasma cells interspersed throughout the disrupted retinal cells. In addition, the choroid was infiltrated extensively. By the final observation day (day 21) the choroid and retina were replaced totally by a thick layer of fibrovascular tissue containing mononuclear cells and multinucleated giant cells.

Eyelids

Lid lesions were not a prominent characteristic of the ocular disease induced by this dose of virus. Herpetic vesicles appeared at the lid margins of some injected eyes (5 of 12) by day 7, and these resolved by day 21. Herpetic lesions developed on the lids of contralateral eyes in only two of eight mice by day 10, and these healed by day 14. The late and transitory appearance of vesicles on the lids of un.injected eyes after intraocular inflammation develops in these eyes is further evidence against an external route of virus spread being responsible for contralateral ocular pathology.

To summarize these findings, ocular disease induced by intracameral inoculation of HSV-1 appeared to involve primarily the anterior segment of the injected eye (cornea, iris, ciliary body). In marked contrast, the ocular disease that developed in the uninjected eyes was characterized primarily by extensive damage to the posterior segment (retina, vitreous, choroid). These contrasting observations suggest that two different pathogenic processes are set in motion following unilateral inoculation of live HSV-1 into the anterior chamber.

To determine if intracamerel virus induced retinal destruction in only contralateral eyes, it was decided to perform the experiments in which recipient mice received 2 × 10^4 pfu HSV-1 inoculated into both eyes. In all of these mice (16 eyes), histologic examination demonstrated that the retinas remained intact as long as 45 days. Figure 3 presents the results of one pair of eyes removed at day 18 PI. The pattern of pathology was identical for both eyes and also appeared identical to that found in single eyes inoculated with HSV-1. An intense keratouveitis was found (Figs. 3A, B), but the retinas remained intact (Figs. 3C, D). The minimal inflammatory infiltrate in the GCL, similar to that observed in eyes injected unilaterally, also was present. These observations suggest that local protective re-

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sponses develop within virus-inoculated eyes and spare the retina from destruction.

The histopathologic picture of retinal destruction in uninjected eyes suggested that a direct viral assault upon that tissue was operative, and, therefore, it became important to determine whether HSV-1 could be identified in uninjected eyes at any time after inoculation of the virus into the contralateral eyes. Panels of BALB/c mice received HSV-1 into the anterior chamber of one eye or subcutaneously (control). At periodic intervals thereafter, eyes were removed, pooled (2-4 eyes/time point), and assayed for the presence of infectious virus by a plaque assay based on the cytopathic effect of virus on monolayers of tissue cultured cells. The results are presented in Table 2. As early as 5 days after AC inoculation, HSV-1 was recovered from uninjected eyes. As expected, virus-injected eyes contained replicating virus through day 10. By day 14 PI, homogenates of both injected and uninjected eyes failed to exhibit replicating virus. No comparable recovery was obtained from eyes harvested from animals infected with HSV-1 subcutaneously. These results confirm that infectious HSV-1 is present in uninjected eyes within a few days of its inoculation into the contralateral eye.

Table 2. Evidence for HSV-1 spread to uninjected eyes

<table>
<thead>
<tr>
<th>Route of inoculation</th>
<th>Virus titer in uninjected eyes (TCID&lt;sub&gt;50&lt;/sub&gt;/eye)</th>
<th>Days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior chamber</td>
<td>2.1 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>5</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>&gt;5 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>7</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
</table>

* Two to four uninjected eyes were pooled and inoculated in 0.5-ml medium. One-tenth-milliliter aliquots were incubated on rabbit skin cell monolayers. At 3–5 days, monolayers were assessed for the presence of cytopathic effects (CPE). Homogenates of eyes that had been inoculated with virus produced CPE on days 5, 7, 10 (1 × 10<sup>5</sup>, >5 × 10<sup>4</sup>, 7 × 10<sup>3</sup> TCID<sub>50</sub>/eye, respectively). Titers of HSV-1 in each eye were determined by the method of Reed and Muench. 31
Discussion

Inoculation of HSV-1 (KOS) directly into the anterior chamber of mouse eyes produces a characteristic pathology. Within 24 hr of intracameral inoculation with this virus, the inoculated eye develops an acute intense inflammatory reaction within the anterior segment, involving the cornea, iris, the lens, and the lining of the anterior chamber itself. This reaction proceeds to destruction of most of these ocular tissues, but with preservation of the retina. Seven days after inoculation of HSV-1 into one eye, inflammatory reactions are appreciated in the contralateral (uninjected) eye. While involvement of the entire uveal tract of the uninjected eye was observed consistently, the intensity of the retinal destructive lesions was extreme. This pattern of ocular pathology, induced by inoculation of HSV-1 into the anterior chamber of murine eyes, resembles in many ways a similar pattern observed when herpes simplex virus has been inoculated into the anterior chamber of rabbit eyes.5

Two general hypotheses have been advanced to explain the ocular lesion following HSV infection. It could be proposed that the pathologic changes observed are all the direct result of the cytopathogenic effect of the virus itself, or it might be that some (perhaps most) of the pathologic changes observed following HSV-1 infection of the eye are secondary to immune responses made against infected tissues, the end-result of which are the destruction of ocular tissues.4 Considering the sequence of events that follows inoculation of HSV-1 in mice, it can be proposed that virus first infects tissues lining the anterior chamber, and then spreads distally and to the contralateral eye. Our histopathologic findings are consistent with localization of viral antigens in rabbit eyes after HSV-1 inoculation into one anterior chamber as described by Pettit et al.33 While the inflammatory changes observed in the contralateral eye may result from a direct cytopathogenic effect of the virus, it is also possible that the contralateral lesions may result from (1) the direct actions of cytotoxic T-lymphocytes or (2) the effect of antigen—antibody complexes that elicit destructive immunologic reactions at the site of virus deposition or infection. Whichever of the two polar hypotheses is correct—direct cytopathogenesis versus immune-mediated destruction—neither accounts for the most extraordinary aspect of the ocular changes that follow HSV-1 injection of the anterior chamber of one eye—namely, the retina of the inoculated eye is preserved, while its contralateral cohort is destroyed completely.

To address this unusual pathologic finding, experiments were conducted in which HSV-1 was inoculated simultaneously into the anterior chambers of both eyes. The finding that the retinas of both eyes were preserved is a striking one and strongly implies that within the inoculated eyes, locally produced protective factors are able to protect the retinal neurons in situ from the cytopathogenic effect of the virus. Based on this observation, we would propose that a new consideration be included in the understanding of the pathogenesis of herpes-induced ocular disease: HSV-1 presented into the anterior chamber elicits within the eye a local protective response, which is able, in some manner, to spare the ipsilateral retina from direct virus-mediated cytopathic effects. Whatever the nature of this local factor (interferon, antibody, suppressor T-cells?), it does not disseminate systemically to afford protection to the retina of the contralateral eye. It will be important to discover the nature of this local protective effect.

Preliminary evidence from experiments utilizing nude BALB/c mice supports a role for T-cell-mediated protection of retinas of inoculated eyes. In the majority of eyes examined from nude recipients, both retinas were severely damaged or completely destroyed, regardless of whether one or both eyes received an inoculation with virus (14/18 injected eyes, three of seven uninjected eyes).

Development of a murine model for HSV-induced ocular disease following anterior chamber inoculation offers many advantages. To the present, a great deal has been learned about the sequellae of HSV-1 injected into the anterior chamber of rabbit eyes. However, the lack of readily available inbred strains of genetically defined rabbits, as well as the paucity of immunologic reagents able to identify lymphocyte subsets and immunogenetic markers in this species, has limited the advance and understanding of the pathogenesis of the disease. In conjunction with our studies on the pathology of this murine model of ocular HSV infection, we have evidence that inoculation of HSV-1 into the anterior chamber of mouse eyes produces a deviant immune response that resembles anterior chamber-associated immune deviation (ACAI D) elicited by alloantigenic tumor cells placed in the anterior chamber.29,34 However, at present, our analysis has not proceeded to the point that we are able to identify which, if any, features of the HSV-1-induced ocular disease result from the participation of an ACAID-like phenomenon. It is possible that destruction of the contralateral retina results from direct infection with the virus, whose spread is thus unimpeded, a spread that might under normal circumstances be prevented by
immune T-cells and/or specific antibody. Studies bearing on this important point are currently in progress.

Key words: Herpes simplex virus type 1, anterior chamber, herpetic ocular disease, mice, pathogenesis

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References