Two Waves of Virus Following Anterior Chamber Inoculation of HSV-1
Solly S. Arherton and J. Wayne Streilein

Following inoculation of herpes simplex virus type 1 (HSV-1) into the anterior chamber of one eye of a Balb/c mouse, a pattern of ocular retinal disease occurs which is characterized by retinal necrosis, disruption of the retinal architecture of the uninoculated contralateral eye, and sparing of the retina of the virus-inoculated eye. Our direct virus culture studies have revealed that, after unilocular anterior chamber inoculation, virus reaches the uninoculated eye in two temporally separate waves. The first wave of virus is detected in the uninoculated eye as early as one day postinoculation (pi), long before virus is found in either of the optic nerves or the brain. The second wave of virus arrives in this eye between 7 and 10 days pi. Sequentially, the path of the second wave of virus appears to move from the injected eye to (1) the ipsilateral optic nerve, (2) the brain, (3) the contralateral optic nerve, and (4) the posterior segment of the uninoculated contralateral eye, suggesting that interocular spread of the second wave of virus after anterior chamber inoculation occurs via neural pathways. Results of histopathologic examinations and virus culture studies suggest that the early wave of virus contributes to the inflammation observed at the angle structures of the contralateral eye 7–8 days pi and that the second wave of virus accounts for the peak virus titer observed on day 10 pi, a peak which coincides with the destruction of the retina of this eye. It is proposed that the first wave is causally related to the development of retinitis, which occurs as the second wave reaches the retina. Invest Ophthalmol Vis Sci 28:571–579, 1987

Inoculation of the KOS strain of herpes simplex virus type 1 (HSV-1) into the anterior chamber of one eye of a Balb/c mouse produces a characteristic constellation of ocular findings, including a destructive inflammatory reaction that develops within the posterior segment of the uninoculated eye, resulting in pan-neurocrisis of the retina 10–14 days postinoculation (pi). The anterior segment of this eye is relatively uninvolved in the inflammatory process. Although the anterior segment of the injected eye is extremely inflamed, there is a characteristic sparing of the retinal architecture in the posterior segment of this eye. While this syndrome was first described experimentally in rabbits by von Szily,1 experimental interest was rekindled only recently. Whittum et al,2 studying immune deviation induced by antigens placed into the anterior chamber of mouse eyes, inoculated HSV-1 into the anterior chamber of one eye, and observed the von Szily phenomenon in the experimental animals. Coincident with the development of retinitis in the contralateral, uninoculated eye, the mice were observed to develop typical anterior chamber associated immune deviation (ACAID), an immune response characterized by suppressed delayed hypersensitivity and production of circulating, neutralizing antibodies to HSV-1. In a critical experiment, HSV-1 was inoculated simultaneously into the anterior chambers of both eyes of normal mice; acute retinal necrosis failed to develop in either eye, indicating that a host defense mechanism, perhaps acting locally, was operative in the protection of the retina of the inoculated eye.

Little is known about the mechanism by which the ipsilateral retina is spared following HSV-1 inoculation into the anterior chamber. There is similarly sparse information concerning the pathogenesis of the retinal necrosis in the contralateral eye. Because previous studies demonstrated that infectious virus was present in the uninoculated eye, it was proposed that the retinal necrosis might be due to a direct cytopathic effect of the virus. If that is the case, one of the important, and as yet unanswered, questions about this murine model of viral retinitis concerns the route (or routes) by which the virus leaves the inoculated eye and spreads to the uninoculated, contralateral eye.

The present studies were designed to provide evidence bearing on the putative viral pathogenesis of the contralateral retinitis. From experiments which ex-
explored the importance of the route and site of inoculation and the mode of virus spread from the inoculation site, we have acquired results that strongly support the hypothesis that at least one route by which virus disseminates to the uninoculated contralateral eye is neural, as opposed to blood-vascular. Moreover, two temporally distinct waves of virus appear to be important in the pathogenesis of the retinitis in this uninoculated eye.

Materials and Methods

Virus

The KOS strain of herpes simplex virus type 1 (HSV-1) was used throughout these experiments. Virus stocks were propagated in Vero cells grown in complete growth medium (CGM) consisting of Dulbecco’s minimal essential medium containing 5% calf serum and antibiotics. An aliquot of passaged virus was titered in 96-well microtiter plates (Costar, Cambridge, MA) following the method of Stalder et al.3 The titers of virus stocks were calculated in TCID₅₀/ml. Virus stocks were frozen in small amounts and stored at -70°C. A new aliquot of titered stock virus was thawed and used for each experiment.

Animals

Female BALB/c mice 6–12 weeks of age (Charles River Laboratories, Inc., Wilmington, MA) were used in these experiments. Mice were kept in a standard laboratory environment and given unrestricted access to food and water. All animal procedures were carried out using chloral hydrate (0.36 mg/gm body weight) or pentobarbital (0.30 mg/gm body weight) as the anesthetic agent and were performed in accordance with the ARVO Resolution on the Use of Animals in Research.

Virus Inoculation

Inoculations of the anterior chamber were performed using previously described methods.4 The anterior chamber of the right eye of each mouse received an inoculum of 1.5 × 10⁴ TCID₅₀ of HSV-1 contained in a total volume of 4 µl. After proptosis and piercing of the sclera of the eye to be injected, intravitreal inoculations were placed laterally into the vitreous body behind the lens. Animals inoculated via the intravitreal route received an equivalent amount of virus 1.5 × 10⁴ TCID₅₀/4 µl as animals inoculated via the anterior chamber route. In scarification experiments, corneal epithelium was abraded using a 30 gauge needle. Virus was dropped onto the cornea and the lids were held together while the eye was massaged gently. Subconjunctival inoculation of HSV-1 was performed by injecting virus into the subconjunctival space. Intravenous inoculations were placed into a tail vein. Subcutaneous inoculations were given in equal amounts in four body sites.5

Preparation of Tissues For Histopathology or Recovery of Infectious Virus

Tissues for light microscopic examination were fixed in phosphate buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Animals were considered to be positive for retinitis if the microscopic architecture of the retina of the uninoculated eye was destroyed by an inflammatory process characterized by the presence of a large number of inflammatory cells, cellular infiltrates, and fibrinous exudate as described previously.2 Tissues for virus titration were collected and stored at -70°C. Tissues to be titered were thawed and homogenized in CGM using a 1.0 ml tissue homogenizer. Each sample of each tissue type was homogenized in the same amount of CGM. One-tenth ml aliquots of the homogenized tissues (either undiluted or diluted serially in CGM) were adsorbed to monolayer cultures of Vero cells at 37°C for 1–2 hr. Following adsorption, the plates of cells were overlaid with CGM containing 0.5% Sea-Plaque agarose (FMC Corporation, Rockland, ME). After 5 days, plates were fixed with buffered formalin and stained with 0.13% crystal violet. Plaques were counted, and the titer was expressed as plaque forming units (PFU)/ml of homogenized tissue.

DNA Extraction

After clinical examination using a dissecting microscope, both the inoculated and the uninoculated eye of each animal were divided into an anterior and a posterior segment using a scalpel. The anterior segment consisted of the cornea and limbus tissue; the posterior segment consisted of the remaining ocular tissue. The lens and optic nerve were removed from each eye prior to homogenization of the ocular tissue. Tissues for DNA extraction were homogenized in 10 mM Tris-HCl, pH 7.4. The cells were lysed in homogenization buffer containing 50 mM EDTA and 1% SDS. The lysate was treated with Proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, IN) at a concentration of 100 µg/ml for 4–6 hr at 50°C.6 The lysate was extracted with phenol:chloroform:isoamyl alcohol (24:24:1) as often as needed until the interphase was clear and finally with chloroform:isoamyl alcohol. Sodium chloride was added to a final concentration of 200 mM, and the DNA was precipitated in two volumes of ethanol. After pelleting, drying, and resuspension, the DNA was quantified spectrophotometrically prior to dot blotting.
DNA Hybridization

The DNA samples to be dot blotted were alkali-denatured and heated to 80°C for 20 min. After neutralization, the samples (0.5–1.0 μg) were dot blotted onto nitrocellulose sheets (Schleicher and Schuell, Keene, NH). After vacuum drying, the nitrocellulose sheets were prehybridized in a solution of 5X Denhardt’s (50 X Denhardt’s = 1% Ficoll, 1% polyvinylpyrrolidone, 1% bovine serum albumin, wt/vol), 5X SSC (1× SSC = 0.01 M NaCl, 0.01 M sodium citrate), 50% deionized formamide (vol/vol) and 100 μg/ml denatured salmon sperm DNA for at least 8 hr at 42°C. Hybridization was carried out in the same solution to which had been added 10 μg/ml Poly A and 1–2 × 10^6 CPM/ml of 32P-labeled denatured DNA which had been nick translated by the method of Rigby9 using a commercially-available nick translation kit (Bethesda Research Laboratories, Inc., Gaithersburg, MD). Nick-translated DNA had a specific activity > 5 × 10^7 CPM/μg. Following hybridization, the nitrocellulose filter was washed under stringent conditions (0.1X SSC, 0.1% SDS, 50°C), dried and allowed to expose x-ray film (Fuji Photo Film Co., Ltd., Japan) in the presence of intensifying screens. After autoradiography, the individual dots were separated and counted in a liquid scintillation counter using a broad counting window for 32P.

Results

Clinical Examination of Uninoculated and Inoculated Eyes

Experiments were conducted to monitor the clinical progression of the HSV-1 infection as it spread from the inoculated to the uninoculated eye following its introduction into the anterior chamber of the former. At intervals after inoculation, the eyes of HSV-1 injected animals were examined grossly using a dissecting microscope. Until 7 days pi, the contralateral eye appeared to be normal; between days 7 and 10 days pi, injection of the circumlimbal vessels and prominence of the iris vessels occurred. At this time, the pupils of most contralateral eyes became hyporeactive to light. Subsequently, the pupils of some eyes became fixed and dilated.

Direct examination of the inoculated eye revealed that inflammation and evidence of virus infection (clouding of the cornea, neovascularization of the cornea) were present in all of the eyes at 3 days pi. As the time after inoculation increased, the inoculated eye showed increasing neovascularization and clouding of the cornea with intense circumlimbal injection and cataractous change of the lens. In injected eyes in which all or part of the pupil could be visualized, pupillary dilatation with accompanying prominence of the iris vessels was observed. By day 14 pi, the cornea of the injected eye was cloudy and extensively neovascularized. Thus, by clinical examination, all injected eyes developed evidence of HSV-1 infection.

The first clinical evidence of virus in the contralateral eye was observed on day 7 pi, and was coincident with the time of arrival of the second wave of infectious virus which was recovered from this eye (see below). In the majority of contralateral eyes, progressive involvement was suggested by the loss of the pupillary reaction to light. With regard to both the inoculated and the uninoculated eye, the clinical gross examination correlated well with the histopathological appearance of the specimen as described below.

Results of Microscopic Examination of Ocular Tissues

In animals inoculated unilaterally with HSV-1 via the anterior chamber route, the predominant histopathological pattern observed in the contralateral eye (65–75% of animals) began within 1 week of virus inoculation. Mild iritis and cyclitis, accompanied by a few inflammatory cells in the anterior chamber were first seen in this eye at 5–6 days pi (Fig. 1A). At this time, the retina of this eye appeared normal. However, 10 days pi, retinas of involved contralateral eyes were observed to be virtually destroyed. Rarely was there evidence of progressive retinal lesions, suggesting that the inflammatory destruction of the retina took place with explosive rapidity. By 14 days pi, retinal destruction was complete, with total disruption of the normal layered architecture as shown in Figure 1C. Neither neurons nor photoreceptor cells were identifiable. Evidence of inflammation persisted in the iris and ciliary body at this time.

In a minority of animals (25–35%), no evidence of retinitis developed in the contralateral eye (Fig. 1D). Importantly, the eyes of these animals also lacked evidence of anterior segment involvement (iritis and cyclitis) as demonstrated in Figure 1B. Circumstantial evidence, therefore, links the early appearance of inflammation at the angle structures with the pan-retinal necrosis that occurs approximately 4–7 days later.

Relationship of the Route and Site of HSV-1 Inoculation to the Development of Retinitis

Based on the early work of von Szily1 and other, more recent investigators2,10–12 who showed that an acute destructive retinitis occurred in the contralateral, uninoculated eye following unilocular anterior chamber inoculation of HSV-1, we performed experiments to determine whether comparable retinitides could also be observed in contralateral eyes of animals inoculated...
Fig. 1. Photomicrographs demonstrating the differences between the two histopathological patterns observed in the uninoculated contralateral eyes. Panels A and C demonstrate the pattern of inflammation in the angle structures (A) and retina (C) of the eye of a mouse exhibiting retinitis of the contralateral eye. Panels B and D show the essentially normal architecture of the angle structures and retina respectively of a mouse without contralateral retinitis. The eyes shown in panels A and B were harvested 7-8 days after unicocular anterior chamber inoculation; the eyes shown in panels C and D were harvested 14 days pi. (X215).

with virus at ocular sites other than the anterior chamber. We conducted histopathologic examinations of ocular tissue harvested from animals inoculated with the KOS strain of HSV-1 via the following routes: anterior chamber (11 animals), subconjunctival (15 animals), corneal scarification (10 animals), and intravitreal (14 animals). For comparison, other animals (6 animals/group) received subcutaneous or intravenous inoculations of the same amount of virus.

A summary of the results of these studies is presented in Table 1. Contralateral retinitis was observed only in eyes of animals inoculated with virus via the anterior chamber and intravitreal routes. In no animal did corneal scarification or subconjunctival inoculation of the KOS strain of HSV-1 result in retinitis in either the uninoculated contralateral eye or the inoculated eye. Neither the intravenous nor the subcutaneous inoculation of live HSV-1 produced a retinitis in eyes of any animal tested. The fact that intravitreal inoculation of HSV-1 also produced retinitis is not germane to the present inquiry and will be considered in subsequent studies.

Route of Virus Spread Following Unicocular Anterior Chamber Inoculation

Following anterior chamber inoculation, experiments were performed to determine when and where virus could be found among relevant neural structures. Such determinations would yield information about when virus escaped from the injected eye and by what possible route(s) the virus could leave the injected eye to gain access to the uninoculated contralateral eye. There are several neural routes by which virus could reach the contralateral eye. These include: (1) the ipsilateral optic nerve through the optic projections, then via crossed fibers to the contralateral optic nerve, (2) the interconnections of the autonomic nerves supplying both eyes and orbital contents, and (3) the sensory portion of the ophthalmic nerve (V1) which is associated with general, nonvisual sensation of the eye and orbit. Experiments were performed to deduce the route of virus spread by examining when and from where infectious virus or viral genomes could be recovered at intervals following unicocular anterior chamber inoculation. The standard dose of HSV-1 was inoculated into the anterior chamber of euthymic Balb/c mice. At intervals after inoculation, three to five animals were sacrificed, and the various tissues assayed for infectious virus or for the presence of viral genomes.

Virus Culture Studies

The eyes, other tissues closely related to the eyes (both optic nerves, both halves of the brain and both

Table 1. Development of retinitis following inoculation of HSV-1 at ocular and extraocular sites

<table>
<thead>
<tr>
<th>Route of virus inoculation</th>
<th>Injected eye</th>
<th>Uninjected eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior Chamber</td>
<td>0/11*</td>
<td>7/11</td>
</tr>
<tr>
<td>Intravitreal</td>
<td>11/14</td>
<td>8/14</td>
</tr>
<tr>
<td>Corneal Scarification</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Subconjunctival</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>Retinitis (any eye)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extravascular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
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* Number of positive samples/total number tested.

Results of experiments demonstrating that retinitis developed only after anterior chamber or intravitreal inoculation of HSV-1. HSV-1 (1.5×10⁶ TCID₅₀) was inoculated into one of several sites as described in Materials and Methods. Fourteen to twenty-one days later, both eyes of the animals were removed, fixed, paraffin-embedded, sectioned and stained. An eye in which the retinal architecture was destroyed by an inflammatory process was considered to be positive for retinitis.
trigeminal ganglia), and distant organs (spleen, kidneys, lungs, liver) from immunocompetent mice infected with the KOS strain of HSV-1 via the anterior chamber route, were homogenized and cultured for infectious virus as described in Materials and Methods. As expected, no infectious virus was recovered from any distant organs (data not shown). Since animals inoculated with virus intravenously did not develop retinitis, the results of the virus culture studies confirm that following unilocular anterior chamber inoculation, virus did not spread to the contralateral eye, even indirectly by a route involving initial vascular dissemination to distant body sites followed by infection of the retina with virus seeded from these sites.

The eyes and nearby extraocular tissues were assayed for the presence of infectious virus to determine how long virus persisted in the injected eye, when virus appeared in the contralateral eye, and if and when the brain and other structures associated closely with the visual apparatus became infected with virus. Virus could be detected in the injected eye during the initial 10–12 days following inoculation of virus using the anterior chamber route (Fig. 2). In the contralateral eye, the maximum virus titers were obtained at day 10 pi (Fig. 2), the time at which maximum destruction of the retina was demonstrated in histopathological sections. However, small but significant amounts of virus could be detected in the contralateral eye as early as 1 day pi (see below).

Following anterior chamber inoculation of the right eye, virus was recoverable from the right half of the brain beginning at day 3 and from the left half of the brain beginning at day 5 pi (Fig. 3). Virus could not be detected by direct viral culture on or after day 10 pi from either half of the brain, indicating that immune clearance had taken place. Virus was present in one of four right optic nerves and in zero of four left optic nerves at days 1, 3, and 5 pi (Fig. 4). Virus could be recovered from the left optic nerve only at days 7 and 10 pi. No virus was present in the right optic nerve by day 10 pi. Small amounts (<1.5 × 10² PFU) of virus were recovered from the left trigeminal ganglia of two of four animals only at day 7 pi (data for trigeminal ganglia not shown). Small amounts of virus (<4.15 × 10³ PFU) could also be cultured from the right trigeminal ganglia between days 3 and 7 pi. No virus was recovered from either trigeminal ganglion of any animal at day 10, 12, or 14 pi.

Hybridization Studies to Localize Virus to Anterior/Posterior Ocular Segments

Since virus culture studies had been performed on the entire inoculated or uninoculated eye, dot blot hybridizations were used to define the location of virus within each eye and to confirm the results of the culture experiments. The results of dot blot hybridizations demonstrate that viral DNA was detected in the anterior segment of the inoculated eye only on days 1

![Graph](image-url)
and 3 pi (Table 2). Although the retinas of the injected eyes remained essentially intact, as judged by light microscopic examination, viral DNA was present in the posterior segment of the injected eyes at day 3 pi. After day 3, virus detection by DNA:DNA hybridization on tissue samples from the posterior segment of the injected eye decreased rapidly. Thus, appearance of virus at the ipsilateral retina without evidence of retinitis indicated that retinal injury must require more than simply the presence of virus.

In the contralateral eye, in agreement with the direct virus culture findings, viral genomes could be detected by day 1 pi. By day 10, the time at which maximum destruction of the retina of the uninoculated contralateral eye takes place, six of nine animals were positive for viral genomes in the posterior segment of the contralateral eye. In agreement with the results of the direct culture studies, viral DNA could be detected in this segment as late as 14 days pi. These results suggest also that the eye of animals from which no DNA was recovered may represent those eyes in which retinal necrosis did not occur.

These results suggest that, following anterior chamber inoculation, HSV-1 arrives at the un inoculated eye in two temporally distinct waves; an early wave which reaches the anterior segment of this eye, and a second (later) wave which gains access to the posterior compartment of the uninoculated eye. The later wave of virus spreads via neural connections involving both optic nerves and the brain. Although circumstantial evidence also indicates neural spread of the first wave of virus, the interocular route of this wave of virus remains to be determined.

**Discussion**

Our experiments to delineate viral spread in the retinitis which occurs in the contralateral eye after inoculation of HSV-1 into the anterior chamber of one eye have yielded several observations. (1). Two temporally separate waves of virus travel to the contralateral eye, an early wave within 24 hr of inoculation and a later wave 7 to 10 days pi. (2). Interocular transport of the second wave of virus is accomplished by a neural pathway involving the central nervous system. (3). Most animals develop contralateral retinitis after un inocular inoculation of HSV-1.

Virus cultures demonstrating that the virus can be detected within the uninoculated contralateral eye at two different times and sites suggest that there are two separate waves of virus which reach this eye. Detection of these two waves was unexpected, and raises the possibility that they may be related to each other and/or to the eventual development of retinitis in this eye. The first wave of virus reaches the contralateral eye within 24 hr of un inocular virus inoculation, a surprisingly rapid transit. Yet the HSV-1 titer in this eye remains at a low level until 7 days pi, when the titer begins to rise rapidly, coincident with the arrival of the
second wave of virus entering via the optic nerve. In view of the timing of its arrival, this first wave of virus is unlikely to undergo more than one cycle of virus replication before it reaches the uninoculated eye. Because of the speed of its arrival and because virus could not be cultured from either optic nerve until much later in the course of the infection, this first wave does not appear to spread via the optic nerves and their interconnections. Even though this first wave of virus reaches the contralateral eye early after infection, the virus appears to replicate rather slowly at the site at which it arrives. Once detected, the virus titer in this eye did not begin to increase until after day 5 pi, a time interval in which replicating virus would have been expected to reach a much higher titer. The consistent observation of focal iritis and cyclitis in the contralateral eye beginning at 1 week pi (preceding the rise in virus titer in this eye) suggests that the first wave of virus may locate in or near the ciliary body.

The second wave which reaches the contralateral eye approximately 7 days pi arrives following viral replication within the substance of the brain and optic nerves as shown by the direct culture results. Following anterior chamber inoculation, this wave involves the sequential spread of virus to the ipsilateral optic nerve by day 3, both halves of the brain by day 5, and finally the optic nerve of the contralateral eye by day 7. Because of the close temporal relationship between the appearance of this second replicating wave of virus and the development of retinitis in the contralateral eye, we suspect that the second wave is responsible for the majority of the virus recovered by direct culture from the contralateral eye during the time at which retinal destruction occurs.

The relationship, if any, between the two waves of virus to the development of retinitis in the contralateral eye is not obvious from the results of these experiments. It is possible that the first wave is unrelated to the second and does not play a role in the devastating contralateral retinitis. A more intriguing possibility is that the limited replication which accompanies the first wave of virus is responsible for the inflammation observed early in the angle structures of the contralateral eye. By disrupting the integrity of the blood-ocular barrier, this early inflammatory process could allow virus-specific immune cells and/or antibodies to enter the intraocular compartment. These immune effectors could then participate in an immune-mediated destruction of the retinal cells infected by the second wave of the virus. Alternatively, the first wave may also allow virus to spread posteriorly to the retina from the ciliary body and contribute to retinal destruction by direct, cell-to-cell extension of the virus.

The precise route(s) by which the virus leaves the inoculated eye to gain access to the uninoculated eye remains incompletely determined. Since HSV-1 inoculated into the anterior chamber induces ACAID, viral antigens must drain directly from the anterior chamber into the blood vascular compartment. While we cannot rule out completely the possibility that HSV-1 escapes the inoculated eye by a vascular route, the fact that intravenously-inoculated virus failed to induce retinitis or colonize ocular structures, makes it extremely unlikely that the intravascular route could be the relevant route by which HSV-1 spreads to the contralateral eye. Our results also suggest that the KOS strain of HSV-1 does not possess a particular tropism for the retina or related ocular tissues, since virus inoculated by neither the subcutaneous nor the intravenous route caused histopathologic evidence of retinal infection. These results are not surprising, since other investigators have shown that herpes virus does not usually cause a viremia and that spread of infectious virus via the blood vasculature is unlikely.

We then considered the following to be possible routes by which the virus could leave the injected eye to gain access to the uninoculated contralateral eye: (1) autoinoculation by such means as scratching or fighting with cage-mates, and (2) via the neural connections between the two eyes through the central nervous system. We believe that spread by the first route did not occur, since autoinoculation from the infected, injected eye to the uninfected, contralateral eye should result in the virus being deposited on the corneal epithelium of the uninoculated eye, followed by infection of the epithelium and stromal layers. Clinical and histopathologic examinations revealed that the epithelium of the uninoculated eye was never involved in an inflammatory process. In addition, other investigators have shown that virus cannot be cultured at days 1–14 pi from the uninoculated contralateral eye of Balb/c mice after HSV-1 infection of scarified corneas. These results, in a system in which early corneal shedding of virus from the inoculated eye might lead to autoinoculation of the uninoculated eye, tends to rule out autoinoculation as the route by which first-wave virus travels to the uninoculated eye after uninoculated anterior chamber inoculation of HSV-1.

Peiffer and coworkers have shown that intracerebral inoculation of HSV-1 into mice leads to bilateral retinitis in approximately 10% of animals. They have demonstrated that virus spreads centrifugally from the site of inoculation in the brain to the eyes by way of neural routes. Our results also suggest that the development of contralateral retinitis following anterior chamber virus inoculation depends on the spread of virus via neural routes. Our virus recovery data suggest that the second wave of virus spreads via neural routes that involve the central nervous system. In the second wave, virus enters first the ipsilateral optic nerve and
gains access to the ipsilateral side of the brain before it reaches the contralateral side. This observation is consistent with previous investigators' observations that not all optic nerve fibers in rodents decussate at the optic chiasm; ie, some fibers remain uncrossed. The second wave of virus appears to gain access to the contralateral optic nerve as a result of infection of the brain, and we postulate that this second wave of virus travels centrifugally down the optic nerve to infect the cells of the contralateral retina. A recent report documenting the extracocular spread of rabies virus from the anterior chamber to the brain and then to the retina suggested that in this system viral infection of the optic nerve occurred several days after virus inoculation, and that virus reached the brain and retina by one or all of several pathways including the oculomotor parasym pathetic nerve, the preopticoretinal pathway, and the ophthalmic nerve.

Our results indicate the route of spread of the second wave of virus is fairly straightforward: virus can be found sequentially in (1) the inoculated right eye (OD), (2) the right optic nerve, (3) the right brain, (4) the left brain, (5) left optic nerve, and (6) almost simultaneously, in the contralateral retina (OS). However, the data suggest that another pathway also exists, one which allows the virus to reach the contralateral eye within 24-48 hr after virus inoculation. The early, first wave of virus appears to be associated with the iritis and cycilitis which are observed in the contralateral eye prior to the development of retinitis and subsequent retinal necrosis. Since the optic nerves and brain were not positive for virus 24-48 hr pi, and since intravascular spread of the virus is unlikely, the consistent histopathological observation of inflammation of the iris and ciliary body tends to support the role of the parasympathetic component of the oculomotor nerve (supplying the ciliary body) in the interocular spread of HSV-1 by the first wave. Experiments to trace the first wave of virus are in progress.

The finding of maximum virus titers in the uninoculated contralateral eye at 10 days pi corresponds to the time at which the most striking histopathological evidence of retinal destruction is observed. Virus detection by dot blot hybridization from the posterior segment of the uninoculated eye also supports a role for viral replication and associated cytopathic processes in the pathogenesis of the retinitis observed in the contralateral eye following unilateral anterior chamber inoculation of HSV-1. It was surprising to discover that virus could also be recovered from the posterior segment of the ipsilateral eye whose retina is spared from the pathologic process. The experimental finding that only one eye develops retinitis although virus is present in both eyes suggests that the presence of infectious virus is not sufficient to account for the uniconal retinal destruction observed in this virus system. The extent to which other virally-associated processes or virus-specific immunological factors contribute to the unique pattern of ocular pathology following anterior chamber inoculation of HSV-1 awaits further investigation.

We found consistently that not all of the animals developed contralateral retinitis in the uninoculated eye after anterior chamber presentation of HSV-1. This result may be a reflection of the Balb/c mouse's intermediate level of natural resistance to the virus, as well as of the fact that the dose given was below that to which animals will succumb when the virus is presented by other routes. The dose of virus which was used in these experiments was selected as the amount of virus which results in reproducible disease in the inoculated eye following anterior chamber inoculation. Our results showed that not all of the contralateral eyes were culture-positive for virus at 1 day pi. If the first wave of virus facilitates the entry of, or potentiates the effects of, the second wave, it is possible that the animals without evidence of contralateral retinitis represent those animals in whom a first wave of virus fails to reach the contralateral eye. Future experiments will attempt to define the relationship of the two waves of virus to each other and to the retinitis in the uninoculated eye.

Key words: herpes simplex virus type 1, retinitis, anterior chamber, mouse, central nervous system

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