Retinitis and Deviant Immune Responses Following Intravitreal Inoculation of HSV-1

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Following anterior chamber inoculation of herpes simplex virus type 1 (HSV-1) into one eye of adult, immunocompetent BALB/c mice, an interesting pattern of ocular pathology and systemic immune responses emerges, characterized by (1) destruction of the contralateral retina with sparing of the ipsilateral retina; and (2) impairment of delayed hypersensitivity (DH) accompanied by intact humoral immunity to the virus. Experiments using animals inoculated via the intravitreal route revealed a different pattern of retinitis in the inoculated and in the uninoculated fellow eye following intravitreal inoculation of HSV-1. The retinitis in the inoculated eye results in localized, focal necrosis with concomitant preservation of the retinal architecture in areas juxtaposed to those in which retinal destruction occurs. The retinitis in the uninoculated contralateral eye is characterized by pan-retinal inflammation and subsequent loss of the architecture of the entire retina. Intravitreally inoculated animals exhibited virus-specific impairment of DH responses to HSV-1 but were capable of producing anti-HSV-1 neutralizing antibody. Impairment of DH response after inoculation of live HSV-1 suggests that intraocular processing of viral antigens occurs such that processed antigens are released systemically in a soluble form. Invest Ophthalmol Vis Sci 28:859–866, 1987

Anterior chamber inoculation of herpes simplex virus type 1 (HSV-1) in adult, immunocompetent BALB/c mice produces a deviant immune response, characterized by suppressed delayed hypersensitivity (DH) along with vigorous neutralizing antibody responses directed at HSV-1 antigens. At the same time, a spectrum of ocular pathologic changes occurs, including acute anterior segment inflammation and destruction in the injected eye accompanied by preservation of the ipsilateral retina, and, paradoxically, a devastating pan-retinitis and subsequent necrosis in the contralateral, uninoculated eye. The relationship, if any, between the unusual systemic immune response to the virus and the extraordinary pattern of ocular pathology remains obscure. Possible explanations for preservation of the ipsilateral retina include the following: (1) infectious virus is unable to gain access to the retina of the injected eye, perhaps for anatomic reasons not yet understood; (2) production of local antiviral factors precludes viral replication in the posterior segment of the injected eye; and (3) suppressed DH prevents an immune mediated response to the virus from damaging the retina via an “innocent bystander” effect. In order to address (1) and (2), direct intravitreal inoculation of infectious virus offers an opportunity to exclude one or more of these possibilities. The results of experiments reported in this article indicate that although direct placement of the virus on and within the retina by intravitreal injection produces a severe focal retinitis, the immune system still responds systemically to the virus in a deviant manner, and contralateral pan-retinitis also is produced.

Materials and Methods

**Virus**

The KOS strain of HSV-1 was used throughout these experiments. Virus stocks were propagated in Vero cells grown in complete growth medium (CGM) 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 and co-workers. The titers of virus stocks were calculated in TCID$_{50}$/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 (Taconic Farms; Germantown, NY), were used in these exper-
imments. Mice were kept in a standard laboratory environment and were given unrestricted access to food and water. All animal procedures were implemented using chloral hydrate (0.36 mg/g body weight) or pentobarbital (0.30 mg/g 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. The anterior chamber of the right eye of each mouse received an inoculum of approximately 1.5 x 10^4 TCID_{50} of HSV-1 contained in a total volume of 4 μl. After proptosing and piercing the sclera of the eye to be injected, intravitreal inoculations were placed laterally into the vitreous body directly behind the lens using a glass micropipette connected to a Hamilton syringe (Reno, NV). Proper placement of the needle was verified by observing the magnified image of the glass pipette through the lens. Animals inoculated via the intravitreal route received an equivalent amount of virus contained in the same volume as animals inoculated via the anterior chamber route. Subcutaneous inoculations were given in equal amounts in four body sites for a total dose of 1-2 x 10^6 TCID_{50}. Intravenous inoculations containing 1.5 x 10^4 TCID_{50} in 0.1 ml were placed into a tail vein.

**Tissue Preparation**

Tissues for light microscopic examination were fixed in phosphate buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin–eosin. Animals were considered to be positive for retinitis if the microscopic architecture of any portion of the retina of either eye was destroyed by an inflammatory process as described previously. Tissues for virus titration were collected and stored at –70°C. At the time of titration, tissues to be titrated were thawed and homogenized in CGM using a 1.0-ml tissue homogenizer (Wheaton Instruments, Millville, NJ). Because all samples of a given type of tissue contained in the same volume as animals inoculated via the anterior chamber route. Subcutaneous inoculations were given in equal amounts in four body sites for a total dose of 1-2 x 10^6 TCID_{50}. Intravenous inoculations containing 1.5 x 10^4 TCID_{50} in 0.1 ml were placed into a tail vein.

In time course experiments, animals were inoculated with virus via the intravitreal route as described in the Materials and Methods section. Animals were killed at intervals after inoculation, and both the inoculated eyes were examined by light microscopy.

**Neutralization Assays**

At the time of killing (10-12 days after inoculation), blood was collected for serum separation. Sera were stored at –70°C before use. Microneutralization assays were performed using previously published methods. Serial, twofold dilutions of serum were incubated with HSV-1 (KOS) for 30 min at 37°C and were then added to 96-well plates that had been seeded with 1.0 x 10^4 Vero cells/well. The 50% endpoint titers for neutralization assays were determined by Reed-Muench calculations as the titer at which 50% of the cell monolayers remained intact.

**Results**

Histopathologic Examination of Inoculated and Uninoculated Eyes

In time course experiments, animals were inoculated with virus via the intravitreal route as described in the Materials and Methods section. Animals were killed at intervals after inoculation, and both the inoculated eyes were examined by light microscopy.
Fig. 1. Photomicrograph demonstrating the focal appearance of the retinitis observed in the injected eye 10 days after intravitreal inoculation of herpes simplex virus type 1. Areas of normal-appearing retinal tissue are adjacent to areas in which the layered retinal architecture has been obliterated completely (×121).

The histopathologic examinations revealed that animals inoculated via the intravitreal route developed retinitis in both eyes within 2 weeks following intravitreal inoculation of one eye. These studies also revealed a difference in the histopathologic appearance between the retinas of the two eyes that was striking. The results of the examinations of the ipsilateral (right) eyes may be summarized as follows. Most of the animals (12 of 14; 86%) developed retinitis in the ipsilateral eye. The ipsilateral retinitis occurred within 7-10 days after inoculation. Initially focal in appearance, the retinal architecture of an area of the ipsilateral eye in some areas was completely destroyed by an intense inflammatory process. These areas of focal destruction and necrosis were directly adjacent to those areas of the ipsilateral retina in which the retinal architecture was largely preserved (Fig. 1). By 14 days after inoculation, the entire retina of the ipsilateral eye had become inflamed and necrotic and was replaced by a fibrocellular scar.

In sharp contrast to the initial focal presentation of the retinitis in the ipsilateral eye was the destructive pan-retinal inflammatory process observed in some of the uninoculated eyes. In slightly more than one half (8 of 14; 57%) of the uninoculated contralateral eyes, there was a pattern of inflammation and accompanying destruction of the  retinal architecture that was similar to the retinal necrosis, which has been observed previously in the uninoculated contralateral eyes of animals following injection of HSV-1 via the anterior chamber route. Six to 8 days after unilucular intravitreal inoculation, the uninoculated eye exhibited a mild anterior uveitis (Fig. 2), which was followed 4-6 days later by total destruction of the retinal architecture (Fig. 3). These findings suggest that at least two patterns of retinitis may be observed after intracameral inoculation of HSV-1: (1) a retinitis in the ipsilateral eye that is initially focal and that may be the result of deposition of virus directly in and onto the retina during the inoculation procedure; and (2) the massive panretinal necrosis that is observed in the uninoculated contralateral eyes of animals inoculated with HSV-1 by either the anterior chamber or the intravitreal route.

Virus Recovery From Inoculated and Uninoculated Eyes

Our previous results have shown that after unilucular anterior chamber inoculation of HSV-1, two separate waves of virus (as defined by two temporally separate peaks of virus titer) reach the uninoculated contralateral eye—an early wave as early as day 1 after inoculation and a later wave at day 10 after inoculation. The arrival of the second wave at day 10 corresponds to the time at which maximal destruction of the retina of the uninoculated eye occurs. Therefore, the later wave has been implicated in the retinal destruction that occurs in the uninoculated eye following unilucular anterior chamber inoculation of HSV-1.

Because the retinas of some of the contralateral eyes of intravitreally inoculated animals were necrotic and appeared to be similar microscopically to the contralateral eyes of the uninoculated animals, the results of the examinations of the contralateral eyes of intravitreally inoculated animals were similar to those of the uninoculated contralateral eyes of animals inoculated with HSV-1 via the anterior chamber route.
lateral eyes of animals inoculated unilaterally with virus via the anterior chamber route, the authors attempted to determine whether virus was also present in the uninoculated contralateral eyes of animals inoculated via the intravitreal route. The presence of virus in the uninoculated eyes would be strong evidence for virus also being responsible at least in part for the destruction of the retina of the contralateral eye in animals inoculated via the intravitreal route. Following intravitreal inoculation, time course experiments were performed in which both the inoculated eye and the uninoculated contralateral eye were harvested from animals on days 1, 3, 5, 7, 10, 12, and 14 after inoculation tissues from three or four animals were collected at each time point. Plaque assays for infectious virus were performed on the homogenized tissues as described in the Materials and Methods section.

As early as 1 day postinoculation, virus was detected in the uninoculated eye (Table 1). The titer of this early wave of virus in this eye was highest at day 1 after
inoculation and decreased steadily until day 7 when the virus titer again began to increase. The second peak of infectious virus recovery occurred at day 10 after inoculation. By day 14 following intravitreal inoculation of HSV-1, all contralateral eyes were negative for virus. The virus recovery studies using samples from uninoculated eyes demonstrated that, similar to the pattern of virus recovery following anterior chamber inoculation of HSV-1, virus reaches the uninoculated contralateral eye at two separate times following intravitreal inoculation. Virus is cleared from the uninoculated eye by 14 days following virus injection.

The amount of infectious virus recovered from the inoculated eye peaked at day 5 after inoculation and declined steadily until day 14 after inoculation, when a small amount of virus was detected in one of four ipsilateral eyes (Table 1).

Previous investigations have demonstrated that two temporally separate peaks of virus titer are detected in the uninoculated contralateral eye following uniocular anterior chamber inoculation of HSV-1. The similarity between the time of recovery of infectious virus in both the uninoculated and the inoculated eye after intravitreal inoculation and the time of infectious virus recovery in both eyes after uniconocular anterior chamber virus injection suggests that the route of spread of virus from the inoculated to the uninoculated eye is comparable whether the virus is placed in the anterior chamber or into the vitreous cavity.

Because only 54% of animals developed retinitis of the contralateral eye following intravitreal inoculation of virus and because all contralateral eyes were positive for virus as early as day 1 after inoculation, these findings would suggest that (1) the early presence of virus in the contralateral eye is not in and of itself sufficient to cause retinitis; and/or (2) the development of retinitis depends on a certain minimum amount of virus being present in the contralateral eyes. In these studies, two of four animals had infectious virus in the uninoculated contralateral eye at day 10 after inoculation, the time at which maximum retinal destruction occurs following either anterior chamber inoculation or intravitreal uninoculated inoculation. A logical conclusion would be that only animals who exhibit a second peak of virus on day 10 following intravitreal inoculation develop retinitis.

### DH Response Following Uniocular Intravitreal Inoculation

Inoculation of HSV-1 into the anterior chamber of one eye of a BALB/c mouse has been shown to induce anterior chamber associated immune deviation (ACAID), the hallmarks of which are a systemic impairment of the ability to make a DH response to the contralateral eye at day 10 after inoculation, the time at which maximum retinal destruction occurs following either anterior chamber inoculation or intravitreal uninoculated inoculation. A logical conclusion would be that only animals who exhibit a second peak of virus on day 10 following intravitreal inoculation develop retinitis.

### Table 1. Recovery of infectious virus from eyes following intravitreal inoculation of herpes simplex virus type 1

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Inoculated right eye (no. positive/total)</th>
<th>Uninoculated left eye (no. positive/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.45, 5.20, 4.54, 4.51 (4/4)</td>
<td>4.86, 3.89, 3.74, 2.45 (4/4)</td>
</tr>
<tr>
<td>3</td>
<td>5.99, 4.99, 3.33, 2.30 (4/4)</td>
<td>3.07, 2.96, 2.70, &lt;1.0 (3/4)</td>
</tr>
<tr>
<td>5</td>
<td>6.17, 5.95, 5.91, 5.78 (4/4)</td>
<td>2.02, &lt;1.0, &lt;1.0, &lt;1.0 (1/4)</td>
</tr>
<tr>
<td>7</td>
<td>4.65, 4.48, 4.41, 4.12 (4/4)</td>
<td>3.54, 2.76, &lt;1.0, &lt;1.0 (2/4)</td>
</tr>
<tr>
<td>10</td>
<td>4.95, &lt;1.0, &lt;1.0, &lt;1.0 (1/4)</td>
<td>5.15, 2.37, &lt;1.0, &lt;1.0 (1/4)</td>
</tr>
<tr>
<td>12</td>
<td>5.08, &lt;1.0, &lt;1.0, &lt;1.0 (1/4)</td>
<td>3.05, &lt;1.0, &lt;1.0, &lt;1.0 (1/4)</td>
</tr>
<tr>
<td>14</td>
<td>3.75, &lt;1.0, &lt;1.0, &lt;1.0 (1/4)</td>
<td>&lt;1.0, &lt;1.0, &lt;1.0, &lt;1.0 (0/4)</td>
</tr>
</tbody>
</table>

* Ocular tissue was removed from four animals at each of the time intervals shown. Tissues were homogenized and assayed for virus by plaque assay on Vero cells. PFU = plaque-forming units.

### Table 2. Impaired delayed hypersensitivity responses in animals inoculated intravitreally with herpes simplex virus type 1

<table>
<thead>
<tr>
<th>Route of priming (day 0)</th>
<th>Virus dose (TCID&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>Challenge dose ultraviolet-inactivated</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(day 8)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Intravitreal</td>
<td>1.5 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>1.0 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>82 ± 10†</td>
</tr>
<tr>
<td>Anterior chamber</td>
<td>1.5 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>1.0 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>36 ± 8†</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>1.0 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>1.0 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>163 ± 10</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td></td>
<td>1.0 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>14 ± 6</td>
</tr>
</tbody>
</table>

* The ear swelling response of each animal was determined by the following formula:

\[
(24 - 0\text{ hr}_{\text{experimental}}) - (24 - 0\text{ hr}_{\text{control}}) \times 10^{-3}\text{ mm}
\]

† Significantly different from subcutaneous group: P < 0.01 or better.
viral antigens while humoral antibody-forming ability remains intact. Experiments were performed to determine if intravitreal inoculation of HSV-1 resulted in an impairment of virus-specific DH similar to the DH deficit which has been observed previously following anterior chamber inoculation of HSV-1. On day 0, groups of experimental animals were inoculated with 1.5 × 10^4 TCID$_{50}$ of HSV-1 via the intravitreal route. Negative control animals received tissue culture medium only; DH positive controls received HSV-1 subcutaneously. On day 8, one ear of each animal received 10 μl of ultraviolet-inactivated HSV-1 equivalent to 1.0 × 10^6 TCID$_{50}$ before inactivation. The other ear of each animal received 10 μl of culture medium as a control for changes in ear thickness owing to nonspecific inflammation caused by manipulation and the injection.

As shown in Table 2, animals inoculated via the intravitreal route showed severe impairment of the DH response to HSV-1, similar in degree to that observed in animals inoculated with HSV-1 via the anterior chamber route. In several experiments, ear swelling responses in intravitreally inoculated animals ranged from 50–62% of the subcutaneously inoculated, DH-positive controls. Animals inoculated via the subcutaneous route developed strong DH responses (positive control). As expected from previous observations, animals inoculated with virus via the anterior chamber route showed impairment of virus-specific DH responses. The impaired DH responses in animals inoculated by either the anterior chamber or the intravitreal route were not significantly different from the values obtained for the negative control animals who had received an inoculum of tissue culture medium alone. Thus, inoculation of HSV-1 by the intravitreal route with a dose of virus that leads to the induction of a DH response when animals are primed with virus by the subcutaneous route leads to impairment of virus-specific DH, which is similar in extent and timing to that observed in animals inoculated via the anterior chamber route.

Table 3. Titer of herpes simplex virus type 1 (HSV-1) neutralizing antibody*

<table>
<thead>
<tr>
<th>Route of inoculation</th>
<th>Virus dose (TCID$_{50}$)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravitreal</td>
<td>1.5 × 10^4</td>
<td>1:8</td>
<td>1:12</td>
<td>1:26</td>
</tr>
<tr>
<td>Anterior chamber</td>
<td>1.5 × 10^4</td>
<td>1:26</td>
<td>1:15</td>
<td>1:39</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>1.0 × 10^6</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>—</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
</tr>
</tbody>
</table>

* Serum samples (collected 10–12 days after virus inoculation) from five to six animals per group were assayed for HSV-1 neutralizing antibody. Results are expressed as mean titers.

Discussion

The authors' experiments to determine the pattern of potential virus-induced pathology, the interocular spread of infections, and the systemic, specific antiviral immune response following intravitreal inoculation of HSV-1 have resulted in several interesting observations. (1) Unlike the sparing of the ipsilateral retina after anterior chamber inoculation of HSV-1, a retinitis occurs in the intravitreally inoculated eye. The lesion in the ipsilateral retina is characterized initially by a focal necrosis that eventually spreads to involve the entire retina, forming a fibrocellular scar. (2) Similar to the results of experiments employing unioocular anterior chamber inoculation of HSV-1, virus reaches the uninoculated contralateral eye of all animals within 1 day following uninoculated contralatetal virus inoculation. This first wave of virus is followed 7–10 days after inoculation by a second wave, which is detected in only one half of the animals. (3) Approximately 50% of the animals inoculated via the intravitreal route develop retinitis in the uninoculated contralateral eye. (4) Virtually all animals inoculated with HSV-1 via the intravitreal route exhibit impairment of virus-specific DH. (5) However, these animals are capable of producing sig-
significant amounts of neutralizing antibody to the virus, a pattern of immune response similar to that observed following unilocular anterior chamber inoculation of HSV-1.

The finding of ipsilateral retinitis following intravitreal HSV-1 inoculation is probably not surprising because during and following inoculation, infectious virus particles would be expected to be placed into direct contact with the retina. The retinitis observed in the inoculated eye was markedly different from the comparatively mild inflammatory involvement of the nerve fiber and ganglion cell layers, which has been observed previously in the ipsilateral eye of animals inoculated via the anterior chamber route. The retinitis seen in the ipsilateral eye of animals inoculated via the intravitreal route was also strikingly different from the inflammatory, pan-retinitis seen previously in uninoculated contralateral eyes after unilocular anterior chamber inoculation of a comparable dose of HSV-1. Following intravitreal inoculation, the ipsilateral retinitis was initially more focal and characterized by areas in which there was intense inflammation and complete destruction of the retinal architecture. The initial focal presentation of the retinitis suggested that early virus replication occurred at sites in which virus was deposited as a result of the intravitreal method of inoculation such as along the track of the inoculating needle. Because intravitreal inoculations pierce all layers of the posterior aspect of the globe, it is also possible that virus was deposited locally in the subretinal space and was able to infect the retina at this site.

Similar to what has been observed previously in animals inoculated with HSV-1 unilocularly via the anterior chamber route, virus spreads quickly (within 1 day of inoculation) to the uninoculated contralateral eye. Virions comprising the early, first-appearing peak of virus apparently did not replicate after reaching the contralateral eye, because the viral titers recovered from representative uninoculated eyes at 3 days after inoculation were uniformly lower than those observed on the first day following intravitreal inoculation of the other eye. The conclusion that the first wave of virus reached the contralateral eye but did not replicate suggests that the first wave reaches a site within the uninoculated eye that is not permissive for or is not well suited for viral replication. Because a mild anterior uveitis is observed in uninoculated contralateral eyes at least 3 days before destruction of the retina of this eye occurs, it is possible that early, low-titered, first-wave virus is responsible for this mild inflammatory process. The fewest number of contralateral eyes of animals (one of four samples) were positive at day 5, and it was on this day that the virus titer from the first wave of virus to reach this eye was the lowest. Approximately one half of animals exhibited a second wave of virus in the uninucleated contralateral eye, the titer of which peaked on day 10 and which corresponded to the time of maximum destruction of the retina. Circumstantial evidence therefore links the presence of replicating virus to the retinitis observed in uninoculated contralateral eyes. The possible relationship of the two waves to each other remains unclear.

These experiments have also demonstrated that, when virus is placed into the vitreous body, neutralizing antibodies are made to the virus, and systemic DH reactivity to the virus is impaired. It has been shown previously that a similar immunologic pattern of response emerges when HSV-1 is inoculated via the anterior chamber route. This pattern of impairment of DH reactivity with concomitant production of neutralizing antibodies to the virus is ACAID. Its occurrence after intravitreal inoculation of HSV-1 suggests that either the intraocular placement of virus is all that is required for suppression of a DH response, or more likely, virus and viral antigens are processed in a similar manner following either anterior chamber or intravitreal inoculation. Other investigators have shown that the introduction of a particulate antigen into the vitreous did not lead to the development of antigen tolerance as assessed by ear swelling experiments. Only soluble antigen placed into the vitreous body led to suppression of DH responses. Based on this reasoning, the results of the experiments reported here can be interpreted to suggest that viral antigens may be processed within the confines of the globe and then released as soluble antigen(s).

These experiments also have shown that virus placed into the vitreous body by a route that of necessity pierces the retina can produce ipsilateral retinitis as well as an impaired DH response to the virus. The results of the intravitreal experiments presented here suggest that under certain circumstances (such as placing the virus into direct contact with the retina) retinitis can be induced in an eye inoculated with HSV-1 via an intraocular route. In addition, it has been proposed that protection of the ipsilateral retina from pan-retinal necrosis following anterior chamber inoculation of HSV-1 is dependent on suppression of the virus-specific DH response. The results presented herein suggest that suppression of DH is not responsible for protection of the ipsilateral retina because ipsilateral retinitis was observed in the presence of systemic impairment of virus-specific DH responses. The possible role of ACAID or of the impairment of the DH response such as occurs following intravitreal inoculation of HSV-1 in the destructive process observed in the contralateral eye is not yet obvious.

Noted added in proof: Metzger and Whittum-Hudson (Metzger EE and Whittum-Hudson JA: The dichotomy between herpes simplex virus type 1-induced ocular pathology and systemic immunity. Invest
have also observed that BALB/c mice develop virus-specific suppression of DH following intravitreal inoculation of the KOS strain of HSV-1.

**Key words:** herpes simplex virus type 1, retinitis, immune response, mouse, vitreous

**Acknowledgments**

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**References**