Immunologic Modulation of Virus-Induced Pathology in a Murine Model of Acute Herpetic Retinal Necrosis

Judith A. Whittum-Hudson and Jay S. Pepose

Unilateral inoculation of herpes simplex virus Type 1 (KOS strain) into the anterior chamber of BALB/c eyes produces an ocular disease with a distinctive differential pattern of retinal pathology. Specifically, the retina of the inoculated eye remains histologically intact, whereas the contralateral retina becomes necrotic. We demonstrate that retinal necrosis in opposite uninjected eyes directly correlates with the presence of herpes simplex viral antigens, whereas the intact retinas of virus-injected eyes are devoid of immunocytochemically detectable viral antigens. Immunosuppression or lack of a thymus results in bilateral retinal necrosis, with positive immunoperoxidase staining for viral antigens in both eyes. We have shown previously that retinal protection in both eyes can be restored to irradiated recipients by adoptive transfer of spleen cells from mice primed by AC injection of HSV. Our results with reconstituted and normal mice suggest that virus-mediated cytopathic effects underlie contralateral retinal necrosis since HSV antigens are localized to areas of retinal necrosis and their presence precedes the local inflammatory response; immunosuppression does not alter the development of contralateral retinal necrosis. They also indicate that ipsilateral retinal preservation reflects T cell-mediated inhibition of viral spread to retinas of injected eyes. Reconstitution of irradiated recipients with AC primed donor cells prevents immunohistochemically detectable virus and retinal necrosis in both eyes. In all experimental groups we failed to detect viral antigens in the absence of retinal pathology. Invest Ophthalmol Vis Sci 28:1541-1548, 1987

The herpes family of viruses can produce retinal necrosis and concomitant encephalitis in a broad spectrum of immunologically competent and compromised hosts. Herpes simplex virus (HSV), for example, has been shown to be a cause of retinitis in neonates,1,2 in acquired immune deficiency (AIDS) victims,3,4 and in organ transplant recipients undergoing immunosuppressive therapy.5,6 Indeed, a recent report indicated that HSV retinitis may also affect non-immunocompromised adults.7 Immune factors that may modulate and control the spread of herpetic retinitis and/or encephalitis are currently ill-defined. We therefore exploited a well-controlled and readily manipulated animal model of acute herpetic retinal necrosis in an effort to better understand the pathogenic mechanisms underlying this disease.

Inoculation of herpes simplex virus Type 1 (HSV-1) into one anterior chamber (AC) of eyes of BALB/c mice produces a distinctive pattern of ocular pathology: virus-injected eyes develop anterior segment inflammation, while the majority (>80%) of retinas are preserved indefinitely. However, the opposite uninjected eyes develop retinal necrosis with-out anterior segment disease. Within 2 weeks these retinas are destroyed and replaced by chronic inflammatory tissue.8 It was previously demonstrated that preservation of the retina of the virus-injected eye is an active T cell-dependent process. Natural or experimentally-induced T cell immunosuppression results in bilateral retinal necrosis, but protection can be restored by immune reconstitution with spleen cells from AC-primed donor mice.9

We report here our studies of the temporal expression of viral antigens in eyes of normal and immunosuppressed mice and the effects of immune reconstitution on virus spread after AC inoculation of HSV-1. Retinal histopathology correlated directly with the detection of viral antigens and preceded the local cellular inflammatory response. The results indicate that virus-mediated cytopathic effects play a primary role in the resultant retinal necrosis, and that retinal preservation in AC-inoculated eyes is an immunologically mediated process which limits the spread of virus.
Materials and Methods

Ocular Infection of BALB/c Mice

Female BALB/c mice (6-8 weeks old), obtained from Charles River Breeding Laboratory (Wilmington, MA), were inoculated with approximately 2 × 10^4 pfu of live herpes simplex virus Type 1 (KOS strain) into the anterior chamber (AC) of one eye as previously described. In some experiments, athymic BALB/c mice obtained from Harlan Sprague Dawley (Indianapolis, IN) were included. Mice were deeply anesthetized with ketamine hydrochloride prior to intracocular inoculation. At varying times after AC inoculation, mice were killed with ether anesthesia and their eyes removed. Eyes were snap-frozen in liquid nitrogen, embedded in O.C.T. (Tissue Tek, Miles, Naperville, IL), and stored at -70°C until sectioning. In some experiments, eyes were fixed in 5% buffered formalin and embedded in paraffin. The ARVO Resolution on the Use of Animals in Research was adhered to in every respect.

Immunoperoxidase Staining For Viral Antigens

Frozen sections were acetone-fixed (5 min) and stained by a modification of the ABC immunoperoxidase staining technique as described by Hsu et al to detect herpes simplex virus Type 1 antigens. Following rehydration in phosphate buffered saline (PBS) and incubation with normal goat serum (1:50), rabbit anti-HSV-1 (MacIntyre VR3; Accurate Chemical, Westbury, NY) at a dilution of 1:500-1000 was applied for 30 min. After three PBS washes, sections were incubated for 30 min with biotinylated goat anti-rabbit IgG (H and L chains) antibody (1:200; Vector Laboratories, Burlingame, CA). Following three washes, the ABC reagent was applied for 45 min, after which the immunoperoxidase reaction was developed with 3-amin-9-ethylcarbazole (AEC; Sigma, St. Louis, MO) in dimethyl sulfoxide diluted in sodium acetate buffer containing 0.01% hydrogen peroxide, until a red reaction product developed. Duplicate negative control slides received a normal antibody a normal rabbit serum known to be negative for HSV-1 antibodies (Accurate). Positive control slides, known to be positive for HSV-1 antigens, were also included in each assay. After developing and washing, all slides were counterstained with Harris' hematoxylin (Harleco; American Scientific Products, McGaw Park, IL), washed, and mounted with Gelvatol (Monsanto, Springfield, MA). Serial sections were Giemsa-stained for detailed histologic examination by light microscopy.

Time Course of Virus Spread Between Injected and Uninjected Eyes

The appearance of viral antigens was followed in both eyes of normal BALB/c mice after unilateral AC inoculation of HSV-1. At days 3, 5, 7, 10, and 14, mice were sacrificed with ether and eyes processed for immunohistochemistry. In two experiments, 3-4 mice were used for each time point.

Reconstitution of Irradiated Mice

To examine the effects of immunosuppression on virus spread, BALB/c mice were sublethally irradiated by exposure to a Cesium-137 source (Gammarcell 40) for a total of 450 R. Twenty-four hours later, 2 × 10^4 pfu of HSV-1 was inoculated into one anterior chamber. In some experiments, irradiated mice were first reconstituted with spleen cells from either normal or HSV-primed BALB/c mice via the tail vein 24 hr after irradiation; 4 hr later, cell recipients received an inoculation of HSV into one AC. HSV-primed cell donor animals received HSV-1 by the AC or SC route 7 days prior to removal of spleen. All recipient mice were killed at varying times after inoculation, and tissues processed for viral localization by the ABC staining method.

Results

Correlation of Ocular Histopathology With Localization of Viral Antigens

Three groups of BALB/c mice were evaluated for histologic signs of retinitis and the presence of viral antigens: (1) normal, (2) sublethally irradiated, or (3) athymic mice. Retinitis was associated with detectable viral antigens in all three groups of mice (Table 1). In the panel of normal mice sacrificed 10-21 days post-inoculation, 15 of 15 virus-injected eyes had normal retinas free of viral antigens. Opposite eyes of these mice stained positively for viral antigens and exhibited varying degrees of retinitis. Irradiated BALB/c mice unilaterally inoculated via the AC with HSV-1 developed retinitis in 100% of both eyes if they were allowed to survive beyond ten days. One hundred percent of those eyes from irradiated mice also demonstrated large amounts of viral antigens. Eight of 13 contralateral eyes from irradiated mice exhibited retinitis by the time of sacrifice (Table 1). In all cases where retinitis developed, viral antigens were present (Fig. 1). The ABC staining technique enabled us to detect as few as two virus-positive cells per section within the ganglion cell layer of contralateral eyes at day 7. Of interest is the observation that the
Table 1. Correlation of viral antigens with retinitis in normal and immunodeficient BALB/c mice*

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Virus-injected Eyes</th>
<th>Uninjected Eyes</th>
<th>Virus-injected Eyes</th>
<th>Uninjected Eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal BALB/c</td>
<td>0/15</td>
<td>15/15</td>
<td>0/15</td>
<td>14/15</td>
</tr>
<tr>
<td>Irradiated BALB/c</td>
<td>13/13</td>
<td>8/13</td>
<td>13/13</td>
<td>8/13</td>
</tr>
<tr>
<td>Athymic BALB/c</td>
<td>13/16</td>
<td>13/16</td>
<td>12/16</td>
<td>7/16</td>
</tr>
<tr>
<td>Athymic BALB/c reconstituted with syngeneic spleen cells</td>
<td>5/5</td>
<td>4/5</td>
<td>3/5</td>
<td>4/5</td>
</tr>
</tbody>
</table>

* HSV-1 (2 x 10^4 pfu) was inoculated into the AC of one eye (ipsilateral) of each mouse on day 0. Ten to 21 days later, surviving mice were killed (or after the first mortality occurred in the immunodeficient groups) and the eyes removed for frozen sectioning. ABC peroxidase staining for HSV antigens was performed: retinitis was determined by histologic examination of Giemsa-stained duplicate sections. One splenic equivalent (5-10 x 10^7 cells) from syngeneic nu/+ BALB/c donors was used to reconstitute athymic mice.

chioroid was negative for viral antigens at all times, even when choroiditis was present.

Typical results of time course experiments determining the spread of viral antigen in normal BALB/c mice are shown in Figure 2. Virus persisted through day 10 in the anterior segment of virus-injected eyes. Herpes simplex antigens were localized primarily in the iris, ciliary body, and the AC cellular exudate (Fig. 2a). Viral antigens were not detected in the intact retinas of virus-injected eyes (Fig. 2b). Only one eye contained viral antigens in its degenerating retina at day 3, and the optic nerve (ON) of this eye also stained positively for viral antigens, suggesting a change in route of virus spread since all other ipsilateral optic nerves were negative at this time point (Fig. 2c). Three of five ON were positive for viral antigens at day 7, although the retinas of those eyes were histopathologically normal and negative for viral antigens. The staining patterns were not identical in the nerves of these three eyes, and varied from staining of only the optic nerve sheath to oligodendrocytes and the nerve fibers. In the anterior segments of opposite uninjected eyes, no virus was observed at any time (Fig. 2d). In contrast, at day 7 a few positive cells were detected in the ganglion cell layer of one uninjected eye. By day 10, 100% of contralateral retinas contained virus and all exhibited varying degrees of retinal pathology (Fig. 2e). Three-quarters of optic nerves were positive for virus at this time, and by day 14 all contralateral optic nerves were positive (Fig. 2f).

Groups of mice which had been sublethally irradiated prior to AC inoculation were examined for virus localization at the same time points chosen for normal mice (Fig. 2, open symbols). Several notable differences were observed between virus-injected eyes of intact and irradiated mice. Virus persisted in the anterior segments of virus-injected eyes from irradiated mice for at least 14 days. While corneal epithelium and stroma were negative for viral antigens, corneal endothelium remained positive for at least 14 days (Fig. 2a). Posterior segments of virus-injected eyes were increasingly positive for viral antigens after day 7 (Fig. 2b). In some of these eyes, only retinal pigment epithelial (RPE) cells were stained for HSV antigens on day 7. Contra lateral retinas never exhibited RPE staining as an early focus of virus. By day 10, 100% of ipsilateral retinas were highly positive for viral antigens. In addition, all optic nerves of virus-injected ipsilateral eyes were positive by day 7. More optic nerves than retinas contained viral antigens at day 7 (80% vs 30%, respectively), suggesting that ipsilateral retinas become virus antigen-positive by contiguous spread into the retina via the optic nerve rather than by viremia (Fig. 2c).

Irradiation appeared to have little effect upon the localization of viral antigens in opposite uninjected eyes: no viral antigens were detected in the anterior segments of those eyes at any time (Fig. 2d). Virus appeared to spread into contralateral retinas of irradiated mice at a somewhat slower rate than observed in normal mice, since only 40% of contralateral retinas were positive for viral antigens at day 10 in irradiated mice (Fig. 2e). In contrast, 100% of contralateral retinas of normal mice were positive at that time. By day 14, all contralateral retinas (uninjected eyes) of both groups showed an extensive distribution of viral antigens.

Effect of Selective Reconstitution on Retinal Infection

Groups of sublethally irradiated BALB/c mice were reconstituted with 5-10 x 10^7 spleen cells (1 splenic equivalent) from donor BALB/c mice which had been primed with HSV-1 by the AC or SC routes. Control irradiated mice received either normal BALB/c spleen cells or no cells at all. The results of two reconstitution experiments are summarized in
Figure 3. Reconstitution with cells from AC-primed donor mice cleared virus from the anterior segments of 75% of injected eyes between days 3 and 10 (Fig. 3a). By day 14, viral antigens were no longer detected in the iris, ciliary body, or anterior chamber of virus-injected eyes. Similarly, at days 10 and 14, retinas and optic nerves of these eyes were histopathologically normal and negative for viral antigens (Figs. 3b–c, 4a). In contrast, the majority of unreconstituted mice or recipients of normal cells exhibited large amounts of viral antigen in the anterior and posterior segments and optic nerves of virus-injected eyes (Fig. 3). Normal syngeneic cells also failed to restore retinal preservation to athymic BALB/c mice (Table 1).

The majority of opposite, uninjected eyes were protected by AC donor cells (Fig. 3d–f). However, normal cells failed to protect any recipient retinas,
Fig. 1. (c) Area of histologically normal retina from same eye shows no evidence of viral antigens. Magnification ×356. All frozen sections were stained by the ABC immunoperoxidase technique and counterstained with hematoxylin.

Fig. 2. Time course of virus spread after AC inoculation. Virus-injected eyes, (a) anterior segment, (b) retina, (c) optic nerve; opposite uninjected eyes, (d) anterior segment, (e) retina, and (f) optic nerve. Three to five mice were used for each time point from 3–14 days after virus inoculation. Presence of viral antigens was determined by immunoperoxidase staining as detailed in the Materials and Methods. Closed symbols, normal BALB/c; open symbols, irradiated BALB/c mice. Hatch marks indicate no irradiated animals were included for day 5.

Fig. 3. Immunocytochemical evidence of virus spread in irradiated BALB/c mice after immune reconstitution. Mice received either AC donor spleen cells (closed circles), normal spleen cells (open triangle), or no cells (closed squares) prior to their AC virus inoculation. Control recipient mice received AC cells but no virus inoculation (open circles). (a–c) Virus-injected eyes, (b–f) opposite uninjected eyes. Four to six mice were included at each time point.
and localization of virus in these eyes did not differ from that observed in irradiated mice, which received no cells, except that at day 14, ipsilateral optic nerves were negative for viral antigens. Lymphoid cells were present in these nerves and may have contributed to viral clearance (manuscript in preparation). In no cases where retinas were preserved were viral antigens detected. In the majority of necrotic retinas, however, antigens were detected (Figs. 3, 4b).

Some sections of ipsilateral eyes in each experimental group contained the ciliary ganglion, lying adjacent to the optic nerve. While the ipsilateral optic nerve was negative for virus in the majority of mice (Fig. 2), the ipsilateral ciliary ganglion, when present, and small nerves presumed to be branches of the ciliary nerve contained large amounts of viral antigen as early as day 3 (Fig. 5). Viral antigens were observed also in the ipsilateral ciliary ganglion of reconstituted
mice at day 3, suggesting that the immune events which result in retinal preservation do not limit viral egress locally from the eye, but act at some extraocular location after viral infection has progressed at least to the ciliary ganglion.

Discussion

Our studies demonstrate a direct correlation between retinal necrosis and the presence of viral antigens as detected by immunoperoxidase staining of frozen and paraffin sections of eyes. These data strongly suggest that contralateral retinal pathology represents virus-induced cytopathology which begins by virus spread from the optic nerve to the inner layers of the contralateral retina and progresses to the outer retinal layers.

We have shown that virus reaches the retinas of opposite eyes 7-10 days after inoculation of virus into the first eye. Previous studies in rabbits\(^1\),\(^2\) failed to detect replicating virus in eyes with necrotic retinas. Failure to detect virus in other models, or at late time points after retinal necrosis has occurred, is possibly related to a loss of cells able to support a productive viral infection or to neutralization of infectious virus. An alternative explanation for the decrease in detectable viral antigens is that following an acute infection, the virus may enter a latent state. Indeed, latent virus has been reactivated from explanted posterior segments of mouse eyes following corneal inoculation,\(^13\) but to date latent virus has not been detected in intact retinas after AC inoculation.

Since the iris, ciliary body, and ciliary ganglion exhibit large amounts of viral antigens at day 3, one route virus takes out of the eye is by the parasympathetic fibers of the ciliary nerve which synapse with the ciliary ganglion cells.\(^14\) These findings are consistent with earlier slit-lamp studies in which we noted loss of the pupillary reflex within 1-3 days after AC inoculation of HSV-1 (unpublished observations).

Previous studies from this laboratory have demonstrated that ipsilateral retinal preservation is an active T cell-mediated process.\(^9\) The lack of detectable viral antigens in intact retinas of virus-inoculated eyes or most ipsilateral optic nerves suggests strongly that T cells prevent the spread of HSV to those retinas after reconstitution with AC-induced donor cells. Most immunodeficient (irradiated or athymic) BALB/c mice developed bilateral retinal necrosis, at which time viral antigens were detected in both eyes. Mice which did not develop bilateral retinal necrosis all showed signs of systemic disease consistent with encephalitis, and presumably were sacrificed before opposite eyes developed complete retinal necrosis. If retinitis was present, failure to detect viral antigens was probably associated with lack of a focus of replicating virus.

The presence of defective interfering (DI) particles in intact retinas of virus-injected eyes has been suggested as a possible mechanism for retinal preservation.\(^15\) However, DI particles are believed to be generated following viral inoculation at a high multiplicity of infection. Because we failed to detect in intact retinas any evidence of viral antigens which would be associated with replicating virus, generation of DI particles within those retinas seems unlikely. Furthermore, DI particles are packaged in nucleocapsids and, if present, would have been detected by the polyclonal anti-HSV antibody used with our immunoperoxidase staining technique.\(^16\)

The reason for the dichotomy between positive optic nerve but negative retinal staining in virus-injected eyes of five unirradiated mice between days 7 and 14 (Fig. 2) is not clear, but may represent a pivotal point in the pathogenic process when systemic immunity is known to be vigorous,\(^17\) and is perhaps able to prevent virus from progressing from the optic nerve to the retina. At day 3, immunohistochemical

![Fig. 5. Detection of HSV antigens in ciliary ganglion. Ipsilateral ciliary ganglion (arrow) after AC inoculation of HSV-1 3 days previously. There is immunohistochemical evidence of large amounts of HSV antigens in the ganglion and a branch of the ciliary nerve (arrowhead) while the optic nerve (ON) is completely negative. Original magnification ×160, hematoxylin counterstain.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933134/ on 06/24/2017)
and histopathologic evidence of model failure could be detected in both retina and optic nerve when it was present. However, the positively-staining optic nerves in virus-injected eyes at days 7–14 were not associated with the presence of viral antigens or pathology in the retinas of these eyes. We hypothesize that the immune system contributes to ipsilateral retinal protection at these later times. In opposite uninjected eyes, optic nerve staining for viral antigens is clearly and consistently associated with the development of retinitis and the presence of viral antigens from day 7 on, and undoubtedly relates to the spread of virus from the brain to the opposite eye.

In summary, these experiments strongly suggest that direct virus-induced cytopathic effects represent the major pathogenic mechanism of retinal necrosis in this murine model of herpetic ocular disease. Retinal necrosis correlates directly with our ability to detect HSV antigens in histologic sections of these eyes, and corroborates our previous isolation of replicating HSV-1 from contralateral eyes. Reduction of the inflammatory cell infiltrate by irradiation does not alter significantly the tempo of contralateral retinal necrosis, suggesting that viral cytopathic effects are primary and precede the onset of the cellular inflammatory response. Following immunosuppression, both retinas become necrotic with abundant expression of viral antigens. The specific effects of immune modulation on the pathways of viral spread from the eye to the central nervous system and back to the contralateral retina are under active investigation. Our current observations, together with those previously reported, suggest that T cell-mediated preservation of virus-injected retinas occurs by inhibiting the spread of infection to these retinas.

Key words: HSV-1 retinitis, acute retinal necrosis, anterior chamber, murine model

Acknowledgments

The authors thank Dr. W. R. Green for helpful discussion, Dr. E. Young and Dr. R. A. Prendergast for manuscript review, and Liddian Lindenmuth for careful manuscript preparation.

References