An Immunogenetic Analysis of Resistance to Herpes Simplex Virus Retinitis in Inbred Strains of Mice

Jay S. Pepose and Judith A. Whittum-Hudson

Specific inbred strains of mice have been shown to vary considerably in their resistance and susceptibility to herpes simplex virus (HSV) infection. We injected $2 \times 10^5$ plaque forming units (PFU) of the KOS strain of HSV-1 intracamerally into one eye of BALB/c, C57B1/6, and FL (BALB/c X C57B1/6) mice. HSV-1 antigens were localized in frozen sections of enucleated eyes at 10 to 14 days post-inoculation. Injected eyes of BALB/c mice showed an anterior uveitis with HSV-1 antigens in the anterior segment and an intact retina free of HSV antigens. The retina of the contralateral uninjected eye was necrotic and contained HSV-1 antigens. In both C57B1/6 and FL mice, HSV antigens were limited to anterior segment structures in the injected eye, whereas, in contrast to BALB/c mice, the contralateral retina appeared histologically normal and contained no viral antigens. The C57B1/6 and FL strains remained relatively resistant to retinal infection even if pretreated with up to 800 Rads of irradiation. The retinas of normal or sublethally irradiated C57B1/6 and FL, but not BALB/c strains, were also resistant to intravitreal injection of HSV. These results suggest that resistance to HSV retinitis is a dominantly inherited trait, which depends only partly upon immunologic factors and may be heavily influenced by the inherent ability of host cells from different murine strains to support a productive viral infection. Invest Ophthalmol Vis Sci 28:1549-1552, 1987

Herpes simplex virus produces a necrotizing retinitis and encephalitis in a wide range of immunologically competent and compromised hosts ranging from neonates to AIDS victims to adults without evidence of immune dysfunction.1,2 The basis of this variation in individual host susceptibility is unclear. In this study, we exploited a well-defined murine model of HSV retinitis in an effort to gain insights into the genetic and immunologic contributions of resistance to viral retinitis.

Pioneering studies by Lopez on the resistance of inbred murine strains to intraperitoneal inoculation with HSV-1 revealed a ten thousand-fold difference in the 50% lethal dose between BALB/c and C57B1/6 mice.4 There has been some controversy, however, regarding the underlying basis of the resistance. In the early study,4 embryo fibroblast cultures of the various strains are equally capable of supporting a viral infection and that the differences in susceptibility are not on a cellular level. One interpretation of these findings was that the differences in resistance to HSV infection inherent to different inbred strains of mice might be associated with their ability to mount an immune response. However, Collier and associates5 obtained disparate results, showing lower levels of viral replication in embryo fibroblast cultures from the resistant C57B1/6 strain, but higher viral titers from embryo fibroblasts from susceptible strains or FL (BALB/c X C57B1/6) hybrids (which were resistant to in vivo infection). Thus, viral replication at the cellular level did not always correlate with resistance to intraperitoneal HSV injection (based upon mortality). Studies assessing the ability of cultured keratocytes from these inbred strains to support HSV replication showed differences in the one-step viral growth curve, suggesting that resistance may be, in part, mediated at the cellular level.6 Others have suggested that resistance may correlate with the IgH-1 allotype and have postulated that this may be in some way linked to the T cell receptor.7

We studied the mechanisms of resistance and susceptibility of specific inbred strains of mice to HSV retinitis in a well-defined murine model.3,8 Briefly, unilateral anterior chamber inoculation of BALB/c mice results in ipsilateral anterior uveitis, but the ipsilateral retina remains histologically intact. In con-
Contrast, the anterior chamber of the opposite uninjected eye remains quiet, but the retina is destroyed within 2 weeks. Previous studies have shown that the preservation of the ipsilateral retina can be ablated by sublethal irradiation or by cyclophosphamide treatment and can be restored by adoptive transfer of T cells. Our recent studies suggest that retinal necrosis is a direct result of productive viral infection rather than an immune-mediated process. In the present investigation, we studied this model of HSV retinitis in BALB/c, C57Bl/6 and the F1 hybrid (BALB/c × C57Bl/6) to: (1) define the resistance and susceptibility of each strain to HSV retinitis; (2) to determine whether resistance is inherited in a dominant fashion; and (3) to assess whether resistance to HSV retinitis is dependent upon systemic immunity or upon other genetically controlled factors.

**Materials and Methods**

**Murine Model of HSV Retinitis**

Female BALB/cJ, C57Bl/6J and F1 (BALB/cJ × C57Bl/6J) mice (Jackson Laboratories, Bar Harbor, ME) were inoculated with $2 \times 10^3$ PFU of HSV-1 (KOS strain) into either the anterior chamber or vitreous. Intraocular injections of 4 μl of viral inoculum were performed through glass needles under direct microscopic observation. If any technical difficulties were encountered, such as clogging of the needle tip or possible pericellular injection, those animals were not included in the analysis. HSV for these experiments was plaque-purified, propagated and titrated on rabbit skin cells. The inoculated mice from each strain were sacrificed between 10 and 14 days post-infection. The enucleated eyes were snap-frozen in liquid nitrogen for serial cryostat sectioning at 6 μm intervals. The distribution of herpes simplex virus antigens at 3, 5, and 7 days post-inoculation, those animals were not included in the analysis. HSV for these experiments was plaque-purified, propagated and titrated on rabbit skin cells. The inoculated mice from each strain were sacrificed between 10 and 14 days post-infection. The enucleated eyes were snap-frozen in liquid nitrogen for serial cryostat sectioning at 6 μm intervals. The studies were conducted in strict adherence to the ARVO Resolution on the Use of Animals in Research.

**Histologic and Immunocytologic Studies**

Every tenth cryostat cut frozen section was fixed and Giemsa-stained for routine histology. Remaining slides at each level were fixed in chilled acetone and stained for HSV antigens using rabbit anti-HSV-1 serum (Accurate Chemicals, Westbury, NY) and the rabbit avidin-biotin complex (ABC) immunoperoxidase method of Hsu et al. Slides were developed using 3-aminoethyl-9-carbazole as the chromogen and were studied by light microscopy.

**Immunosuppression by Sublethal Irradiation**

Based upon our determination of the 50% lethal irradiation dose for each strain of mice, C57Bl/6 and F1 strains of mice were subjected to a total of 800 Rads of irradiation in two divided doses. BALB/c mice received 550 Rads in a single dose. The next day, animals were inoculated with $2 \times 10^3$ PFU of HSV intracamerally or intravitreally, in separate groups. These groups of mice were sacrificed between 10 and 14 days and the eyes were processed for HSV antigens and histology, as described above.

To determine whether the irradiation schedule was immunosuppressive, delayed type hypersensitivity (DTH) to herpes simplex virus was assessed in irradiated and non-irradiated groups of mice from each of the three strains. Groups of irradiated and non-irradiated BALB/c, C57Bl/6 and F1 mice (five mice per group) were given $2 \times 10^5$ PFU of herpes simplex virus subcutaneously (1 day following irradiation in experimental groups receiving irradiation). Separate groups of control mice of each strain did not receive a priming virus inoculation. Six days later, both hind footpads of each mouse were measured with a Mitutoyo engineer's micrometer. After measurement, $10^6$ PFU of ultraviolet-irradiated (inactivated) virus in 0.05 ml of medium were injected subcutaneously into one hind footpad. The other footpad provided the negative control and received 0.05 ml of medium alone. Twenty-four hours later, both footpads were measured and the difference in footpad size was used as a measure of DTH reactivity. Results were calculated as change in footpad size = (24 hr – 0 hr) Experimental – (24 hr – 0 hr) Control $\times 10^{-4}$ inches. Results from each group were compared using a two-tailed student t-test.

**Results**

**Intracamerlal Inoculation of HSV-1**

The distribution of herpes simplex virus antigens 10 to 14 days following intracameral inoculation into the right eye is presented in Table 1. C57Bl/6 and F1 (BALB/c × C57Bl/6) mice are resistant to herpes virus retinitis, even if immunosuppressed with 800 Rads of gamma irradiation prior to inoculation of HSV-1. In addition to the late time points presented in Table 1, eyes of four mice were processed for herpetic antigens at 3, 5, and 7 days post-inoculation using the avidin-biotin immunocomplex technique, and were devoid of detectable herpes simplex antigens. In contrast, inoculation of BALB/c mice results in herpetic infection of anterior segment structures of the inoculated eye (corneal endothelium, anterior chamber, iris, lens, ciliary body), whereas the ipsilateral retina remains histologically intact and free of HSV-1 antigens (Table 1). The contralateral retina is necrotic and contains HSV antigens, which are readily detected by the ABC immunoperoxidase method. Irradiation of BALB/c mice prior to HSV
inoculation results in bilateral herpetic retinitis. As seen in Tables 1 and 2, for the three strains of mice tested using either the intracameral or intravitreal routes of inoculation, there exists a one-to-one correlation between the detection of herpes simplex antigens in the retina and histologic evidence of retinitis.

To determine if the irradiation schedule employed in our study was functionally immunosuppressive, delayed type hypersensitivity (DTH) to HSV-1 was assessed in irradiated and non-irradiated mice of each of the three strains in a separate series of experiments. In non-irradiated mice of each strain, a positive DTH test was obtained to HSV antigens following subcutaneous priming. In contrast, none of the three irradiated groups demonstrated a positive DTH response compared to its non-irradiated counterpart of the same strain or to the unprimed control of the same strain ($P < 0.02$, student t-test). These results document the suppressive effect of the irradiation regimen on DTH against HSV antigens in our system.

**Intravitreal Inoculation of HSV-1**

Intravitreal inoculation of HSV-1 into the right eye of BALB/c mice results in ipsilateral herpetic retinitis, with HSV antigens readily detected (Table 2). When BALB/c mice are sublethally irradiated prior to intravitreal HSV-1 inoculation, HSV antigens are detected in both the anterior segment and vitreous, retina and choroid of the injected eye, as well as in the retina of the contralateral eye. In contrast, C57Bl/6 and F1 mouse strains remain relatively resistant to herpesvirus retinitis, even following irradiation-induced immunosuppression (Table 2). In addition to the late experimental time points presented in Table 2, eyes of four mice from each strain were studied 3, 5, and 7 days post-inoculation and were devoid of detectable herpes simplex antigens using the avidin- biotin immunocomplex technique. The anterior segment structures that were positive for viral antigens in the right eye of two of the C57Bl/6 mice and three of the F1 mice were the lens and/or ciliary body, which may have been inadvertently directly inoculated with HSV by the needle tract passing into the vitreous cavity.

**Discussion**

The results of this study indicate that C57Bl/6 and F1 mice are strongly resistant to HSV-1 retinitis following intracameral or intravitreal inoculation and that this is a dominantly inherited trait. Furthermore, these strains remained relatively resistant to retinal infection despite radiation-induced immunosuppression. In this study, we used ten times more virus in the HSV inoculum than in our previously described model of retinitis to stress the system and test the degree of resistance to infection. We chose to study animals between days 10 and 14 post-inoculation for several reasons. First, our preliminary studies demonstrated that no viral antigens were present at earlier time points in the F1 or C57Bl/6 strains. Second, 100% of the BALB/c retinas expressed viral antigens and were grossly necrotic by day 14 post-inoculation. By choosing days 10 to 14 and evaluating the retinas by both histologic and antigenic criteria, we felt certain that we would not miss retinitis in the F1 and C57Bl/6 strains that was simply delayed in its time course as compared to the BALB/c mice. In addition, impression cytology of posterior segments from C57Bl/6 and F1 mice at 3 weeks post-infection was free of herpesvirus antigens (unpublished studies), again indicating that what we interpret as resistance in the C57Bl/6 and F1 strains is not simply a delay in the time course of viral retinitis.

Studies by Stulting and associates have demonstrated that one-step viral growth curves determined in tissue culture using keratocytes from the various strains correlated well with resistance to corneal infection. This finding, coupled with the apparently minor contribution of the immune system to resistance observed in our studies, suggests that the basis of genetic resistance to HSV retinitis may depend heavily on the ability of each strain to support a productive viral infection at a strictly cellular level.
An alternative explanation for our results is that the specific modality chosen for immunosuppression (irradiation) does not act upon the type of immune cell responsible for resistance. For example, previous studies by Johnson and others have shown that macrophages, which are relatively radioresistant, may play an important role in the pathogenesis and dissemination of HSV infection.

Our data show a dichotomy between the mechanisms affecting the spread of herpes infection in the resistant (F1 and C57Bl/6) versus the sensitive (BALB/c) strains. Whereas radiation-sensitive immune mechanisms play a seemingly minor role in contributing to resistance of C57Bl/6 or F1 mice, immune suppression of the sensitive BALB/c strain results in bilateral retinitis following both intracameral and intravitreal inoculation. We are currently studying the various routes of virus spread from the anterior chamber of the inoculated eye (eg, superior cervical, ciliary and trigeminal ganglia and brain) to determine the anatomic site(s) where viral infection may be blocked in the resistant strains. In addition, we are studying whether different anatomic routes are involved in the irradiated and non-irradiated BALB/c mice, as well as in irradiated mice following immune reconstitution.

Our finding that resistance to HSV retinitis is a dominantly inherited genetic trait with only a seemingly minor role played by the immune system, may explain the sporadic occurrence of HSV retinitis and encephalitis in such a broad spectrum of both immunologically competent and compromised humans.

**Key words:** viral retinitis, herpes simplex virus, uveitis, immunogenetics, resistance

**Acknowledgment**

The excellent technical assistance of Mr. Joseph A. Kessler, II is gratefully acknowledged.

**Table 2. Localization of HSV-1 antigens and histologic retinitis following intravitreal inoculation of HSV-1**

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Pre-treatment regimen</th>
<th>Inoculated* eye</th>
<th>Histologic retinitis injected eye</th>
<th>Uninjected* eye</th>
<th>Histologic retinitis uninjected eye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AS†</td>
<td>PS</td>
<td>AS†</td>
<td>PS</td>
</tr>
<tr>
<td>BALB/c</td>
<td>None</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>0/3</td>
</tr>
<tr>
<td>BALB/c</td>
<td>Irradiated</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>0/6</td>
</tr>
<tr>
<td>C57Bl/6</td>
<td>None</td>
<td>2/9</td>
<td>2/9</td>
<td>2/9</td>
<td>0/9</td>
</tr>
<tr>
<td>C57Bl/6</td>
<td>Irradiated</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>F1</td>
<td>None</td>
<td>0/6</td>
<td>0/6</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>F1</td>
<td>Irradiated</td>
<td>3/11</td>
<td>6/6</td>
<td>3/11</td>
<td>0/6</td>
</tr>
</tbody>
</table>

* Results are reported as number of HSV antigen-positive eyes/number of eyes tested. Eyes from mice given 2 × 10^5 pfu of HSV-1 were tested for HSV antigens between 10 to 14 days post-inoculation.

† AS = Anterior Segment; PS = Posterior Segment.
‡ F1 (BALB/c × C57Bl/6).

**References**