Chorioretinal Disease Patterns in Congenic Mice following Intraocular Inoculation with HSV-1

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Disease patterns and immunologic parameters were studied employing inbred and Igh-1 disparate congenic mice to determine the role of host genetics and Igh-1-linked gene products in the von Szily model of viral chorioretinitis. Following intracameral inoculation of $1.5 \times 10^4$ PFU HSV-1 (KOS), 100% of BALB/c (Igh-1a), 62% of A/J (Igh-1c) and none of the C57BL/6J (Igh-1d) inbred mice developed contralateral necrotizing chorioretinitis. Multigenic differences between inbred mice prohibit conclusions about the specific role of Igh-1-linked immune regulation in this model. In order to more exactly define Igh-1-specific restriction of HSV-1-mediated chorioretinitis, Igh-1-disparate, congenic BALB/c mice were studied following both anterior chamber and intravitreal inoculation protocols. Anterior chamber inoculation resulted in contralateral retinal necrosis in 75% of BALB/c (Igh-1*) mice, 30% of C.AL-20 (Igh-1d) and 5% of the C.B-17 (Igh-1d) congenic mice; all strains showed ipsilateral retinal sparing. Following intravitreal inoculation of HSV-1 a similar restricted disease pattern was found in contralateral eyes. Contralateral chorioretinitis developed in 30% of BALB/c, 15% of C.AL-20 and 6% of C.B-17 mice. Ipsilateral disease, however, was found in all murine strains. These disease patterns developed despite equivalent suppression of systemic DTH and equivalent RPE permissivity to viral replication. These data demonstrate that host genetics strongly regulates contralateral HSV-1-mediated chorioretinal disease patterns by a mechanism unrelated to the development of systemic suppression of DTH and specifically support a dominant role for gene products linked to the Igh-1 locus in the immunomodulation of ocular disease.


The von Szily method of unicocular intracameral inoculation of herpes simplex virus has recently been adapted to a murine model of HSV-1-mediated chorioretinitis.1,2 Studies to date have shown that following the inoculation of HSV-1 into the anterior chamber of one eye of a BALB/c mouse, the virus travels via neuronal pathways to gain access to the contralateral eye, producing a necrotizing chorioretinitis with relative ipsilateral retinal sparing. Intravitreal injection of virus, in contrast, produces both ipsilateral and contralateral chorioretinitis.3 While the exact mechanism(s) responsible for these observations are not entirely known, a unique set of acquired, HSV-specific cellular immune responses develops following inoculation and is implicated in the pathogenesis of the von Szily model.4-6 Specifically, a strong T suppressor cell response is observed and is thought to play an important role in the diminished systemic delayed-type hypersensitivity reaction following intracameral HSV-1 presentation. Murine strains not generating HSV-specific suppression of DTH, termed anterior chamber-associated immune deviation (ACAID), typically develop no disease or only ipsilateral disease with contralateral retinal sparing.6,7 A role for the immune system in disease generation is further supported by studies in immunocompromised mice (nude, gamma-irradiated or cyclophosphamide-treated BALB/c mice) that show a loss of ipsilateral retinal protection and a bilateral retinal necrosis following unicocular HSV inoculation unless the animal is reconstituted with anterior chamber-primed spleen cells.8 In contrast, other investigators support a nonimmune, viral-induced pathogenesis in this model of infectious chorioretinitis.9,10

An important advantage of the murine model is the availability of genetically defined animals. We have previously demonstrated the usefulness of such definition in a keratitis model of HSV-1 infection where we employed BALB/c congenic mice, disparate at the Igh-1 locus on chromosome 12.11 A significant association was found between Igh-1 phenotype

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and the generation of an immune keratopathy. Gene products linked to the Igh-1 region appear to modulate disease through disparate T cell subset recruitment, and not via differences in trigeminal ganglionic latency or keratocyte permissivity.\textsuperscript{12} Susceptible mice demonstrated high T helper to T suppressor ratios while resistant mice had an augmented T suppressor population in cornea and conjunctiva. In order to determine whether the Igh-1 region similarly influences chorioretinitis, we investigated clinical and histologic disease patterns, systemic DTH responses and retinal pigment epithelial cell (RPE) permissivity in HSV-resistant C.B-17, HSV-intermediate C.AL-20 and HSV-susceptible BALB/c congenic mice.

Materials and Methods

HSV Simplex Type 1 Cultivation

HSV-1 strain KOS was obtained from Dr. David Knipe (Harvard Medical School, Boston, MA) and passed twice in Vero cells (American Type Cell Collection, CCL 81). Virus for all experiments was produced from infected Vero cell monolayers as described previously.\textsuperscript{13}

Animals

A/J (Igh-1\textsuperscript{a}), C57BL/6J (Igh-1\textsuperscript{b}) and BALB/cByJ (Igh-1\textsuperscript{a}) inbred mice were obtained from Jackson Laboratories (Bar Harbor, ME). BALB/c congenic, C.AL-20 (Igh-1\textsuperscript{b}) mice breeding pairs were obtained from Dr. Alfred Nisonof (Brandeis University, Waltham, MA) and bred in microisolators mounted in a ventilated animal rack in our animal facility. BALB/c congenic, C.B-17 (Igh-1\textsuperscript{a}) mice breeding pairs were provided by Dr. Charles Sidman (Jackson Laboratories). All animals were handled in accordance to the ARVO Resolution on the Use of Animals in Research.

Intracameral HSV-1 Inoculation

The different mouse strains were inoculated with 1.5 \times 10^4 PFU of KOS strain HSV-1 either in the anterior chamber (AC) or in the vitreal cavity (VC). After intraperitoneal phenobarbital (0.30 mg/g) and topical proparacaine anesthesia, animals were placed under an operating microscope and, following paracentesis, were injected with 5 \mu l of the appropriately diluted virus either AC (through the cornea) or VC (via proptosing and piercing sclera) through a 33 gauge needle connected to a 50 \mu l Hamilton syringe.

Clinical Observation and Analysis

Following intracameral HSV-1 challenge, animals were evaluated under an operating microscope for both anterior segment inflammation and posterior segment inflammation. Anterior segment inflammation was defined as a dilated pupil, enlarged and tortuous iris vessels and cell and flare within the anterior chamber obscuring clear details of the iris. Posterior segment inflammation effects included blurring of fundus detail, dulling of the normally bright red reflex from the retinal and choroidal vasculature, or obvious retinal opacification.

Histopathology

In all experiments, clinical disease was confirmed by routine histologic techniques as described previously.\textsuperscript{9} Briefly, animals were killed by anesthetic overdose 10 days following intracameral challenge. The eyes were removed bilaterally under an operating microscope and fixed in Karnovsky’s fixative (1% paraformaldehyde, 1.25% glutaraldehyde, in 0.2 M sodium cacodylate buffer) for 24 hr at 4°C. The tissue was rinsed in buffer after fixation, dehydrated through ascending concentrations of ethanol, infiltrated with glycol methacrylate solution overnight, and then embedded in LKB Historesin (LKB Produkter AB, Bromma, Sweden). Sections 2 \mu m thick were cut using a Sorvall JB-4 microtome, and stained with hematoxylin and eosin for histopathologic study. The eyes were analyzed for the presence of anterior segment inflammation, retinitis and choroiditis.

Delayed-Type Hypersensitivity

Delayed-type hypersensitivity (DTH) was determined via standard footpad swelling assay as performed in our laboratory.\textsuperscript{14} Briefly, following the various intracameral challenge protocols, one hind footpad received 10^6 erstwhile PFU of UV-inactivated HSV-1 strain KOS in 0.05 \mu l. The other footpad received an equal volume of control Vero cell supernate. Twenty-four hours later, footpad swelling was measured with a Fowler engineer’s micrometer. Naive mice and positive controls (mice inoculated subcutaneously with 1 \times 10^6 PFU HSV-1) were foot-pad-challenged and measured in a similar fashion. All measurements were performed in a masked fashion. Persistent suppression of DTH was calculated by comparing mean DTH values following simultaneous intracameral and subcutaneous priming.

HSV-1 Replication in Cultured Retinal Pigment Epithelial Cells

Methods for isolation and culture of murine RPE were adapted from techniques for rat RPE culture described by Edwards.\textsuperscript{15} Briefly, eyes from 6- to
8-day-old mice were enucleated, placed in Ca2+-enriched balanced salt solution (BSS), and then incubated in a trypsin (1 mg/ml) and collagenase (70 units/ml) solution. Eyes were dissected in culture medium consisting of F-10 defined medium, 20% (v/v) fetal bovine serum and antibiotics. The anterior segment was separated from posterior eyecup, the neural retina removed and RPE sheets teased gently from Bruch's membrane using a microdissecting knife. RPE sheets were dissociated in a trypsin solution (1 mg/ml) and the resulting cell suspension spun at 70 g for 5 min. Cells were then plated in 24-well plates at a density of 50–100,000 cells/well. Cells were incubated at 37°C in the culture medium described above.

RPE cells were allowed to acclimate to culture conditions for 12 hr before infection with virus. At this time, cells from one well were counted, and the remaining wells were infected with HSV-1 (KOS strain) at an MOI = 1. After incubation for 1 hr with virus, cells were washed and fed with culture medium. At 24, 48 or 60 hr post-infection, cells from each well were harvested and subjected to three freeze–thaw cycles. Samples were assayed for infectious particles on Vero cell monolayers using a standard plaque assay.

### Results

Strain-specific differences in susceptibility to chorioretinitis following anterior chamber inoculation have been reported previously by our laboratory employing A/J (Igh-1e), BALB/c (Igh-1a) and C57BL/6J (Igh-1b) inbred mice. While 100% of BALB/c mice (N = 16) developed contralateral necrotizing chorioretinitis after intracameral inoculation of 1.5 × 10⁴ PFU HSV-1 (KOS strain) at an MOI = 1. After incubation for 1 hr with virus, cells were washed and fed with culture medium. At 24, 48 or 60 hr post-infection, cells from each well were harvested and subjected to three freeze–thaw cycles. Samples were assayed for infectious particles on Vero cell monolayers using a standard plaque assay.

#### Table 1. Chorioretinitis frequency following anterior chamber inoculation of HSV-1 strain KOS in Igh-1 congenic mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ipsilateral</th>
<th>Contralateral</th>
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<tbody>
<tr>
<td>BALB/c (N = 20)</td>
<td>5%</td>
<td>75%</td>
</tr>
<tr>
<td>C.AL-20 (N = 20)</td>
<td>10%</td>
<td>30%</td>
</tr>
<tr>
<td>C.B-17 (N = 20)</td>
<td>5%</td>
<td>5%</td>
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Histologic evidence of contralateral chorioretinitis could be found in 31% of BALB/c, 14% of C.AL-20 and 6% of C.B-17 mice (Fig. 2). However, in contrast to anterior chamber inoculation, 62–67% of the ipsilateral eyes also developed a destructive chorioretinitis in all murine strains (Table 2).

These observations demonstrate that gene products encoded by determinants close to the Igh-1 complex modulate the development of contralateral disease following unicocular intracameral HSV-1 challenge but do not significantly influence ipsilateral disease course.

#### Delayed-Type Hypersensitivity Reaction

Simultaneous intracameral and subcutaneous inoculation with HSV-1 results in suppression of delayed-type hypersensitivity (DTH) reaction in all three Igh-1-disparate BALB/c congenic mice when compared to mice primed subcutaneously with HSV-1. There was no significant difference noted between the murine strains (Table 3). Despite different disease patterns, AC and VC inoculation routes resulted in similar suppression of DTH in these mice.

#### RPE Permissivity-Inbred and Congenic Murine Strains

RPE from BALB/c and C57BL/6J mice were infected with HSV-1 at an MOI = 1, and triplicate monolayers were assayed for virus at 24, 48 and 60 hr post-infection (Fig. 3). At each time point, RPE from the two inbred strains showed equal permissivity to virus, as expressed in PFU produced per RPE cell. The results indicate a plateau in RPE permissivity occurring between 24 and 48 hr post-infection.

RPE from BALB/c, C.AL-20, and C.B-17 congenic mice were infected in vitro at an MOI = 1, and triplicate monolayers assayed for virus at 24 hr post-infection (Fig. 4). The RPE from these three strains were equally permissive to HSV-1.

#### Discussion

Murine strain specific susceptibility to HSV infection has been shown for several models of virus-me-
dialed disease. Work in our laboratory and others has established that at least two gene loci are involved in these resistance patterns.\textsuperscript{11,17} In a herpes simplex keratitis (HSK) model we have demonstrated that the Igh-1 region dominantly restricts corneal disease severity. This resistance is not manifest through innate differences in keratocyte permissivity to HSV-1 replication but may be effected through regulation of antibody production or cell-mediated immune responses. Immunohistologic studies have shown that susceptible mouse strains (BALB/c and C.AL-20) have high Lyt 1.2 to Lyt 2.2 lymphocyte ratios in
Table 2. Chorioretinitis frequency following intravitreal inoculation of HSV-1 strain KOS in Igh-1 congenic mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ipsilateral</th>
<th>Contralateral</th>
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</thead>
<tbody>
<tr>
<td>BALB/c (N = 16)</td>
<td>62%</td>
<td>31%</td>
</tr>
<tr>
<td>C.AL-20 (N = 14)</td>
<td>64%</td>
<td>14%</td>
</tr>
<tr>
<td>C.B-17 (N = 15)</td>
<td>67%</td>
<td>6%</td>
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Little information is available on host genetic restriction of herpes simplex-mediated chorioretinitis. We have reported a murine strain-specific pattern of chorioretinitis in inbred mice, which has recently been confirmed by other investigators.\(^7\)\(^10\)\(^11\) In this communication, we further define a role for Igh-1-linked gene products in modulating contralateral chorioretinitis disease severity in two distinct models of intraocular challenge. Anterior chamber inoculation results in ipsilateral protection and VC challenge produces ipsilateral disease in all BALB/c congenic mouse strains. In contrast, a strong Igh-1 restriction can be noted in contralateral eyes. These data suggest that the Igh-1 region modulates the late contralateral ocular milieu and inflammatory events rather than the early ipsilateral virus–host interactions.

It is interesting to note that despite different disease patterns, all three Igh-1 congenic murine strains have identical RPE permissivity. Investigators have postulated from permissivity studies a strong role for non-immune, viral factors in HSV-mediated ocular disease.\(^10\)\(^18\) Although it appears that different inbred mice have distinct cellular permissivity to viral replication, Igh-1-disparate congenic mice have identical keratocyte and RPE permissivity and therefore permissivity would appear to play no role in their different disease patterns found in vivo. Intrinsic permissivity of other ocular tissues such as sensory retina and uvea is not well defined and is currently being investigated.\(^19\)

Kielty et al have postulated that contralateral retinitis develops as a result of HSV-1-specific suppression of DTH in susceptible mice.\(^7\) C57BL/6J mice fail to develop systemic suppression of DTH and are resistant to contralateral retinitis while susceptible DBA/2 mice develop both suppression and contralateral disease. The ability to generate DTH following AC inoculation was felt to be protective. In this communication we present data that distinguish contralateral disease from systemic DTH responsiveness.

Table 3. HSV-1-specific suppression of DTH following intracameral inoculation of HSV in Igh-1-disparate BALB/c congenic mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Percent suppression footpad swelling</th>
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<td></td>
<td>Experiment</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>#1</td>
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<td>#3</td>
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<td>Contralateral</td>
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*P < 0.05 via student t-test. Percent suppression of DTH comparing footpad swelling from control mice primed SQ alone (N = 8 per group) with mice challenged simultaneously with intracameral and SQ HSV-1 (N = 8 per group).

C.B.-17 mice suppress DTH as effectively as BALB/c mice yet do not develop retinitis. These data suggest that systemic suppression of DTH responsiveness (ACAID) is a separate immunologic phenomenon that is unrelated to the mechanism of Igh-1 restriction and specifically contralateral disease generation.\(^16\) This dichotomy is further supported by investigators demonstrating a strong local ocular protective mechanism in the ipsilateral eye unrelated to systemic immunity.\(^7\)\(^8\)\(^20\) Specifically, an abrogation of ipsilateral retinal sparing is noted when animals are inoculated via VC or translimbal routes and when mice are pretreated with Cytoxan despite strong suppression of systemic DTH.\(^8\)\(^20\) In other experiments, bilateral AC inoculation results in no retinal disease in either eye in spite of HSV-specific suppression of DTH.\(^7\) Alternative Igh-1-linked mechanisms perhaps regulating local ocular or regional immunologic mi-
RPE PERMISSIVITY AT 24 hr P.I. - CONGENIC MICE

Fig. 4. RPE permissivity in congenic mice. RPE cells from each strain were infected with HSV-1 in vitro and assayed for infectious virus particles 24 hr later.

lieu or other arms of the immune system appear to be important in modulating contralateral HSV-1-mediated chorioretinitis.

Key words: Igh-1 locus, immunogenetics, mouse, herpes simplex virus (HSV), chorioretinitis, infection

Acknowledgments

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References