Herpes Simplex Virus Type-1 Strain Influence on Chorioretinal Disease Patterns following Intracameral Inoculation in Igh-1 Disparate Mice

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We have previously shown that the Igh-1 locus on chromosome 12 of the mouse influences contralateral disease patterns in the modified von-Szily model. Following intracameral injection with $1.5 \times 10^4$ PFU of HSV strain KOS (HSV-KOS), 75% of BALB/cByJ (Igh-1a), 30% of C.AL-20 (Igh-1d) and 5% of C.B-17 (Igh-1b) mice develop contralateral chorioretinal necrosis. In contrast, Igh-1 congenic mice do not develop contralateral chorioretinal necrosis following anterior chamber inoculation of the same dose of an HSV-KOS mutant lacking surface glycoprotein-C (HSV-GC-KOS). Similarly, injection of wild type HSV strain mP (HSV-mP) into the anterior chamber of susceptible BALB/cByJ mice induces destructive contralateral chorioretinitis whereas injection of the same dose of the mutant HSV strain MP (HSV-MP) lacking surface glycoprotein-C does not induce a destructive contralateral chorioretinitis. In addition, higher doses of HSV-mP inoculum abrogate the Igh-1-associated contralateral chorioretinal protection seen in C.B-17 mice; protection is restored in C.B-17 mice at lower HSV-mP doses that still cause contralateral chorioretinitis in BALB/cByJ mice. These data demonstrate the influence of the viral isolate on the modified von-Szily model and specifically the requirement for the presence of glycoprotein-C on the surface of HSV for the development of contralateral destructive chorioretinitis.

Inoculation of herpes simplex virus type-1 (HSV-1) (KOS strain) into the anterior chamber of one eye of BALB/c mice results in destructive contralateral chorioretinitis with severe ipsilateral iridocyclitis and relative sparing of the ipsilateral retina. Although many theories exist as to the mechanism of this phenomenon that was first observed in rabbits by von-Szily in 1924 and in mice by Whittum-Hudson et al in 1984, the exact mechanism(s) behind the sparing of the ipsilateral retina and the destruction of the contralateral retina is unknown. In order to further define this phenomenon we employed different HSV-1 viral isolates and HSV-1 mutants that differ basically in the presence or absence of the major surface glycoprotein-C in the modified von-Szily model. Glycoprotein-C has been shown by Glorioso et al to be the immunodominant antigen for HSV-1-specific memory cytotoxic T-lymphocytes and by Diefenbach et al to play an important role in the generation of HSV-specific suppression of delayed hypersensitivity reactions (DTH) found in anterior chamber-associated immunodeviation (ACAID). Our results strongly suggest a correlation between the presence of glycoprotein-C and the development of contralateral chorioretinitis in the modified von-Szily model.

Materials and Methods

Experimental Design

The right eyes of the experimental mice were inoculated with one of the viral isolates. The mice were followed clinically every other day for 10 days and then killed by ether overdose. The eyes were removed and processed for histopathology. Separate experiments were conducted in which mice were killed 24 hr, 48 hr, and 10 days after anterior chamber inoculation, and the contralateral (left) eyes were harvested and homogenized for standard viral titration on Vero cell monolayers.

Virus

HSV-1 KOS, mP, MP were obtained from Dr. David Knipe (Harvard Medical School, Boston, MA), HSV-GC-KOS was obtained from Dr. Joseph Glorioso (University of Michigan Medical School,
Ann Arbor, MI) and passed twice in Vero cells. Infected Vero cell monolayers were harvested when a 4+ cytopathic effect was noted. The infected cells were freeze-thawed three times and centrifuged at 1500 g. Supernatants were aliquoted and stored frozen at −70°C. Aliquots were selected at random and assayed using a standard plaque assay technique on Vero cells as described previously. Titres were verified in triplicate.

**Vero Cell Culture**

Vero cell monolayers were maintained in 75 cm flasks (Falcon Plastics, Fisher Scientific Inc., Pittsburgh, PA) using Minimum Essential Media (MEM) with Earle's Salts containing 10% fetal bovine serum (FBS) (Grand Island Biological Co., Grand Island, NY), 0.58 mg/ml L-glutamine (GIBCO), 25 μg/ml Fungizone (Flow Laboratories, Mclean, VA), 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO). Confluent Vero cell monolayers were trypsinized and plated onto six-well, 9.62 cm² tissue culture plates (Linbro, Flow Laboratories, Mclean, VA) for viral plaque assays.

**Animals**

Igh-1 congenic, HSV-1-susceptible BALB/cByJ (Igh-1a), and resistant C.B-17 (Igh-1b) mice, 6–8 weeks old, males and females, were obtained from Jackson Laboratories (Bar Harbor, ME). Sex- and age-matched mice were used in all experiments. Animals were handled in accordance with the ARVO Resolution on the Use of Animals in Research and NIH guidelines and were housed in microisolators within a VR-1 laminar flow isolation unit (Lab Products, Inc., BioMedic Corporation, Rochelle Park, NJ).

**Inoculation**

Animals were anesthetized with ether. Aqueous humor was drained from the right anterior chamber by paracentesis with a glass needle. The anterior chamber of the right eye was then inoculated with HSV using a 33-gauge needle on a 50 μl Hamilton syringe under binocular microscopy. Each eye was inoculated with 1.2 × 10⁵ PFU of HSV-KOS or HSV-GC-KOS mutant contained in a total volume of 10 μl of Minimum Essential Medium (MEM) (Gibco). Pilot studies were performed using virus titers of HSV-mP and MP ranging from 1.5 × 10⁴ PFU to 0.5 × 10⁵ PFU in 5 μl of MEM to determine the optimal challenge dose for contralateral chorioretinitis, ipsilateral chorioretinitis and encephalitis frequencies.
fixed in Karnovsky’s fixative (1% paraformaldehyde, 1.25% glutaraldehyde, in 0.2 mol/l sodium cacodylate buffer) for 24 hr at 4°C before rinsing in buffer and dehydrating through ascending concentrations of ethanol. The globes were then infiltrated with glycol methacrylate solution overnight and then embedded in LKB Historesin (LKB Produkter AB, Bromma, Sweden). Two micron thick sections were cut using a JB-4 microtome and were stained with hematoxylin and eosin (H&E) for histopathology study. Ipsilateral chorioretinitis was defined as the presence of diffuse or focal chorioretinal destruction. Contralateral chorioretinitis was defined as the presence of any combination of chorioretinal necrosis (focal or diffuse), vitreous cellular infiltration and retinal edema (Figs. 3–5).

Recovery of Replicating Virus

Mice were killed 24 hr, 48 hr and 10 days after anterior chamber inoculation using ether overdose. The contralateral (left) eyes were harvested and homogenized in 1 cc of MEM. The iced homogenate was freeze-thawed three times, centrifuged, plated onto Vero cell monolayers and incubated at 37°C. The monolayers were examined daily for 5 days for the presence of cytopathic effects typical of a productive viral infection. Aliquots were saved at −70°C for plaque assay determinations by previously described methods.14

Results

Chorioretinal Disease Patterns following Intracameral Inoculation of HSV-KOS or HSV-GC-KOS

All mice developed an ipsilateral iridocyclitis following anterior chamber challenge with either HSV-KOS or HSV-GC-KOS. Ipsilateral chorioretinitis was found in 10% of BALB/cByJ and 0% of C.B-17 mice when challenged with HSV-KOS. Contralateral chorioretinitis incidence was 65% in BALB/cByJ, and 0% in C.B-17 mice (Fig. 6). These data conform with our previously reported results.15 In contrast, none of the eyes injected with HSV-GC-KOS or the noninjected contralateral eyes in either of the congenic mouse strains developed biomicroscopically or histopathologically detectable chorioretinal disease.

Chorioretinal Disease Patterns following Intracameral Inoculation of HSV-mP or HSV-MP

Initial pilot dose-response studies in the susceptible BALB/cByJ and the resistant C.B-17 mice found HSV-mP to be too lethal when the inoculum was the same as that employed for HSV-KOS; severe encephalitis prevented observation of the evolution of the chorioretinitis model. At the lowest HSV-mP dose tested (0.5 × 10^2) none of the mice (0/5) developed ipsilateral or contralateral chorioretinitis in either mouse strain.
Different results were obtained when higher doses of virus were tested. At $0.1 \times 10^3$ PFU, 75% (6/8) of contralateral and 40% (2/5) of ipsilateral eyes in BALB/cByJ mice developed chorioretinitis; 12.5% (1/8) contralateral and 0% (0/8) ipsilateral C.B-17 eyes developed these findings (Figure 7A). At $0.5 \times 10^4$ PFU, 100% of eyes (7/7 ipsilateral and 9/9 contralateral) of BALB/cByJ mice developed chorioretinitis; 14% (1/7) of ipsilateral and 86% (6/7) of contralateral eyes of C.B-17 mice did so at this dose (Fig. 7B). The highest HSV-mP dose tested was $1.5 \times 10^4$ PFU; most of the mice died of severe encephalitis before day 10 after inoculation (see encephalitis data below). Therefore no further experiments where conducted using this virus dose.

Similar studies were also carried out for HSV-MP.
using the same doses that were used for HSV-mP (see above). The optimal dose chosen for further experiments was \(0.5 \times 10^4\) PFU. Severe encephalitis was obtained with higher doses of this GC<sup>−</sup> mutant virus. At \(0.5 \times 10^4\) PFU, 16% (2/12) of BALB/cByJ mice developed ipsilateral chorioretinitis and 8.3% (1/12) developed contralateral chorioretinitis. None of the C.B-17 mice (0/12) developed chorioretinitis in either eye (Fig. 7C). These results are remarkably different from the results obtained with the parent viral isolates HSV-KOS and HSV-mP.

**Biomicroscopy**

Biomicroscopic examinations of right and left eyes were performed after intracameral inoculation of all viral isolates in order to compare the severity of ipsilateral (injected) and contralateral (noninjected) disease patterns prior to enucleation of the eyes. Ipsi-
eral iritis could be detected as early as 24 hr after intracameral inoculation of all viral isolates in all mice studied; it reached maximal severity by day 3 and was maintained through day 10. The severity of ipsilateral iritis varied with virus strain tested. The mildest ipsilateral iritis was that observed after inoculation of HSV-GC'KOS with an average grade of 1.5 for both BALB/cByJ and C.B-17. Next in severity was the ipsilateral iritis observed with HSV-KOS with an average grade of 2. HSV-MP produced average iritis grades of 3 and 2.5, and HSV-mP produced the most severe iritis with average grades of 4.0 and 3.5, respectively. At the lower dose of 0.1 × 10³ PFU of HSV-mP the difference in grading between BALB/cByJ and C.B-17 mice increased (3.5 vs. 2.5).

The earliest detectable contralateral disease (dilated nonreactive pupils) in either mouse strain was seen on day 3 after inoculation with HSV-mP. The earliest detectable contralateral disease after intracameral inoculation of HSV-KOS was at 7 days. HSV-MP did not produce clinically obvious contralateral disease before day 9.

**Virus Cultures**

Experiments were designed in the BALB/cByJ mice in order to determine whether glycoprotein-C-deficient HSV mutant strains are capable of reaching the contralateral eye. HSV-KOS was used as control. Virus could be isolated in 71% (12/17) of contralateral eyes 10 days after intracameral inoculation of HSV-KOS (average plaque assay titer = 5 × 10³ PFU/cc). Fifty-three percent (8/15) of the mice injected with HSV-MP (average plaque assay titer = 2 × 10³ PFU/cc), and 13% (2/15) of those injected with GC'KOS (average plaque assay titer = 3 × 10² PFU/cc) had virus in the contralateral eye (Fig. 8). None of the cultures from contralateral eyes (0/5) were positive at the earlier time points (24 or 48 hr) post-intracameral inoculation of HSV-KOS, MP, or GC'KOS.

**Encephalitis**

Different patterns of encephalitis were obtained using the experimental virus isolates at various doses. HSV-KOS and its glycoprotein-C-deficient mutant at the tested dose of 1.2 × 10⁴ PFU were found to be non-neurovirulent; none of the mice inoculated intracamerally developed encephalitis. HSV-MP and mP were found to be highly neurovirulent. All the experimental BALB/cByJ and C.B-17 mice (100%) developed severe encephalitis after intracameral inoculation of HSV-MP at 1.5 × 10⁴ PFU. Approximately 50% of the mice died between days 7 and 10 after intracameral inoculation. At 0.5 × 10⁴ PFU inoculum 66.7% (6/9) BALB/cByJ developed moderate encephalitis while 30% (3/10) C.B-17 developed mild encephalitis.

HSV-mP was found to be even more neurovirulent, with 100% of BALB/cByJ and C.B-17 developing severe encephalitis with an inoculum of 0.5 × 10⁴ PFU. After intracameral inoculation of 0.1 × 10³ PFU a different pattern of encephalitis was observed, similar to the pattern of chorioretinitis: 75% (6/8) of BALB/cByJ mice developed moderate encephalitis and 12.5% (1/8) developed severe encephalitis. In contrast, 12.5% (1/8) of C.B-17 mice developed mild encephalitis.

**Discussion**

In 1924, von-Szily first described the phenomenon of contralateral retinitis in rabbit eyes after ipsilateral inoculation of HSV.² This model was adapted to BALB/c mice by Whittum-Hudson et al in 1984, who described sparing of the ipsilateral retina and destructive contralateral chorioretinitis.¹ We subsequently reported on the influence of the Igh-1 locus on the development of contralateral chorioretinitis and showed that, just as in the HSV-1 keratitis model, there is a dramatic influence of the Igh-1 and/or closely linked genes on the susceptibility of mice to develop necrotizing contralateral chorioretinitis.

Roizman and Norrild stressed the importance of HSV surface glycoproteins on the host immune
responses and Carter et al. reported on the importance of HSV-specific glycoproteins in T cell-mediated lysis of HSV-infected cells. HSV-1-induced corneal epithelial and stromal disease patterns also seem to be influenced by viral glycoprotein secretion and can be modified by anti-glycoprotein antibodies.

To our knowledge, the effects of HSV-1 isolates varying basically in the expression of glycoprotein-C on the development of the modified von-Szily model have not been previously reported.

Our data show significantly different disease patterns after intracameral inoculation of wild-type glycoprotein-C-intact HSV-1 strains as compared to their glycoprotein-C-deficient mutants. Whereas the wild-type strains produced contralateral chorioretinitis in 65–100% of contralateral eyes, 0–16% of contralateral eyes were found to be diseased using the glycoprotein-C-deficient mutants as the infecting virus.

The absence of contralateral chorioretinitis could be explained by the inability of the HSV-GC-KOS mutant to gain access to the contralateral eye. This cannot provide a complete explanation, however, since our experiments showed that GC-KOS gets to the contralateral retina in at least 13% of cases. Certainly other mechanisms must explain the lack of disease with the glycoprotein-C-deficient mutant MP since 53% of contralateral eyes without pathology harbored this virus. It is possible that the lack of glycoprotein-C on infected retinal cells limits cytotoxic lymphocyte (CTL) recognition and subsequent lymphocyte-mediated injury in the contralateral eye. This hypothesis is supported by the report of isolation of CTL from contralateral retinas and by evidence reported by Glorioso et al. suggesting that glycoprotein-C is the immunodominant antigen for HSV-1-specific memory cytotoxic T-lymphocytes. Dix has also suggested that glycoprotein-C plays a critical role in HSV-induced humoral responses in vivo.

Alternatively, the absence of suppression of DTH responses (ACAID) after anterior chamber inoculation of glycoprotein-C-deficient HSV isolates, as reported by Diefenbach et al, might play an important protective role against contralateral destructive chorioretinitis. The possible role of active DTH responses in protecting contralateral eyes has previously been addressed by several authors. Atherton et al reported the absence of contralateral chorioretinitis after intracameral inoculation of HSV-2 with a concomitant strong virus-specific DTH response in BALB/c mice. Kielty et al studied the von-Szily model in two inbred strains of mice and found the mouse strain that developed a vigorous DTH response had significantly less contralateral disease. Streilein et al suggested that the induction of ACAID plays an important role in the retinal pathology in the modified von-Szily model. Hayashi et al showed the absence of contralateral retinitis in light-deprived mice after intracameral inoculation of HSV. Concomitantly, these mice failed to develop ACAID and developed a vigorous HSV-specific DTH response.

It is conceivable that the absence of CTL responses and the presence of an active DTH response can act concomitantly, dependently or independently, along with other unknown mechanisms to protect the contralateral eye in this model. The phenomenon of virus isolation in histologically normal eyes is supported by data (unpublished, Atherton, collaborative study) showing positive viral cultures of contralateral eyes in resistant C.B-17 mice after ipsilateral inoculation of HSV-KOS. This phenomenon was also described by Streilein. He was able to culture virus from histologically normal eyes after intracameral HSV-1 and HSV-2 inoculation. This clearly indicates that the presence of replicating virus is not sufficient to induce a destructive chorioretinitis.

The data presented in this report illustrate the strong influence of HSV-1 isolate on the modified von-Szily model. Specifically, our experiments show that herpes simplex virus surface glycoprotein-C plays an important role in the development of the modified von-Szily model. Further investigations are being conducted in our laboratory in order to understand the mechanisms responsible for this phenomenon.

Key words: herpes simplex virus-1, glycoprotein-C, chorioretinitis, congenic mice, von-Szily

References


