The Severity of Herpes Simplex Viral Keratitis in Mice Does Not Reflect the Severity of Disease in Humans

James R. Rinne, Shohla Z. Abghori, and R. Doyle Stulting

Four herpes simplex type 1 virus (HSV) isolates were selected from patients with mild ocular disease and four from patients with severe ocular disease on the basis of the number of epithelial recurrences, presence or absence of stromal disease, visual acuity, and the need for corneal transplantation. The scarified right corneas of 20 BALB/c mice were inoculated with each low-passage HSV isolate (1.0 × 10⁷ plaque-forming units/ml) and examined three times per week for 2 weeks for the presence and severity of epithelial and stromal disease. The eight individual virus isolates differed with respect to the incidence of dendritic disease (P < 0.001), the severity of dendritic disease (P < 0.001), the incidence of stromal disease (P = 0.002), and the severity of stromal disease (P = 0.001) they produced in the mouse. The severity of disease was compared for the two groups of viruses: (1) those that had caused mild disease in their human hosts and (2) those that had caused severe disease. There were no statistically significant differences in the severity or incidence (44 versus 43 animals, respectively) of dendritic disease or stromal disease (27 of 80 animals in each group) between the two groups. These data suggest that the naive BALB/c mouse model of acute HSV keratitis after topical ocular inoculation does not reflect clinically significant differences in the severity of human HSV keratitis that might be caused by variations in the virus genome. Invest Ophthalmol Vis Sci 33:268–272, 1992

It has been hypothesized that strain-specific virus factors are more important than host factors in determining the pattern of herpetic ocular disease in humans. It has been shown that the course of experimentally produced herpes simplex virus type 1 (HSV) keratitis in rabbits is influenced by the infecting virus strain. A defined region of HSV DNA that varies among virus strains has been found to affect the disease pattern. In the inbred mouse model, virus-determined factors also regulate the pattern of HSV keratitis. Differences in the capacity of two HSV strains to replicate in the trigeminal ganglion of its host and spread into the brain has been shown to be determined by nucleotide sequences in the gene for DNA polymerase. Other studies report that susceptibility to HSV ocular disease is influenced by the host genome. The Igh-1 locus on chromosome 12 has been found to control the characteristics of experimental murine HSV ocular disease, and other non-H-2 genetic loci affecting HSV disease characteristics also seem to play a role. However, the relative importance of virus- and host-related factors in humans remains to be determined.

We investigated the possible role of viral factors in determining the pattern of herpetic ocular disease in humans by infecting BALB/c mice with human isolates of HSV and observing the correlated in disease patterns between humans and mice infected with the same virus strain. By using an inbred mouse model, host genetic factors were constant. Therefore, any variation in disease patterns in the animal could be attributed to differences in the viral isolates rather than differences in the host.

Materials and Methods

Virus

By reviewing clinical histories, we selected four HSV isolates from patients with mild HSV ocular disease characterized by, at most, two epithelial recurrences, the absence of stromal keratitis, a good final visual acuity (20/40 or better) not requiring keratoplasty, and unilateral disease. All had been followed for at least 1 year after the most recent recurrence. We also selected four HSV isolates from patients with severe HSV ocular disease characterized by more than three epithelial recurrences, severe stromal keratitis,
and visually significant scarring (20/200 or worse) requiring penetrating keratoplasty (Table 1). Two of the patients with severe disease had bilateral HSV keratitis. These patients met all the criteria for severity in the eye from which the cultures were obtained. No patient in either group showed any evidence of systemic immunologic abnormalities.

The viruses were grown and maintained in African green monkey (Vero) cells. When the cytopathologic effect was 3+ to 4+, the cells were frozen, thawed, and sonicated. Cell debris was removed by centrifugation and stored at −70°C. Stock virus was titrated by plaque assay in Vero cells as previously described.12 Each isolate was passed only once before use in our experiments.

Animal Inoculation

The animals were inoculated by a method previously described.6 Briefly, 4–8-week-old BALB/c female mice were obtained from Jackson Laboratory (Bar Harbor, ME) and allowed to acclimatize to their new environment for at least 1 week. Mice with preexisting ocular abnormalities (determined by slit-lamp examination) were excluded from the experiments.

Under general anesthesia, the corneal epithelium of the right eye of each animal was scratched gently with a 25-gauge needle, using six crisscross strokes. A sterile cotton-tipped applicator then was dipped into the viral suspension (1.0 × 10⁷ plaque-forming units/ml) and rubbed on the cornea for 15 sec. All inoculations were done similarly by one individual who was masked to the source of the viral isolate. Inoculations were rotated (one animal for each isolate in turn), and all inoculations were completed within 3 hours to minimize any experimental variation that might occur because of inoculation timing or storage of the virus on ice. The animals were marked by ear punch so that the course of disease in each individual animal could be followed.

Three times each week for 2 weeks the mice were examined by a masked observer with a slit lamp modified to provide a vertical optical path for small animal observation (Marco Ophthalmic, Inc., Jacksonville, FL) using white and cobalt blue-filtered light after instillation of fluorescein (0.1 mg/ml) in saline. The number, size, and location of dendrites was recorded for each eye. Each dendrite was given a numeric value for its size: 1, length less than one quarter of the corneal diameter; 2, length greater than one quarter but less than one half of the corneal diameter; or 3, length greater than one half of the corneal diameter. The numeric values then were added for all dendrites to obtain an epithelial severity score for each eye. The eyes also were graded on a scale of 0–4 for stromal keratitis, uveitis, and vascularization. Stromal keratitis was classified as follows: 0, none; 1, mild infiltrate or edema; 2, infiltrate sufficient to obscure fine iris detail; 3, dense infiltrate obscuring most iris detail; or 4, dense infiltrate totally obscuring view of anterior chamber structures.6 The mice were examined daily for evidence of systemic disease, and deaths were recorded. The death rates (from anesthesia or presumed encephalitis) were 20%, 15%, and 5% for animals inoculated with isolates 21, 29, and 18. There were no deaths among animals receiving the other isolates.

Two separate experiments were done. In each, ten animals were inoculated on the same day with each viral isolate. The results were equivalent, and data from both experiments were combined. The animals were handled in accordance with the ARVO Resolution on the Use of Animals in Research.

Statistical Analysis

Because the percentage of animals with active keratitis may be misleading if mortality is high or keratitis is transient, the cumulative percentage of animals developing keratitis was used to compare groups (cumulative number of animals with keratitis/number of animals inoculated). The two-tailed chi-square, Kruskal-Wallis, and Wilcoxon rank-sum tests were used to

Table 1. Disease pattern in humans caused by each isolate

<table>
<thead>
<tr>
<th>Severity of disease in the human</th>
<th>Isolate</th>
<th>Epithelial recurrence</th>
<th>Stromal disease</th>
<th>Bilateral involvement</th>
<th>Last vision</th>
<th>Postoperative recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>18</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>20/25</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>20/40</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>20/25</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>20/23</td>
<td>−</td>
</tr>
<tr>
<td>Severe</td>
<td>41</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>20/300</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>5</td>
<td>−</td>
<td>−</td>
<td>HM*</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>19</td>
<td>+</td>
<td>−</td>
<td>20/400</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>9</td>
<td>+</td>
<td>−</td>
<td>20/400</td>
<td>+</td>
</tr>
</tbody>
</table>

* HM = Hand motions.
determine the significance of differences among groups. The smallest difference between the two groups that could be detected with an 80% power also was calculated.13,14

Results

The viral isolates produced epithelial and stromal keratitis in BALB/c mice as previously described.6 The patterns of disease by the various isolates (Figs. 1, 2) differed significantly with respect to the incidence of dendrites \( (P < 0.001, \text{by chi-square test}) \), the severity of dendrites \( (P < 0.001, \text{by Kruskal-Wallis test}) \), the incidence of stromal keratitis \( (P = 0.002, \text{by chi-square test}) \), and the severity of stromal keratitis \( (P = 0.001, \text{by Kruskal-Wallis test}) \).

Five of eight isolates produced epithelial disease above the mean of 55% (Fig. 1). Of these, three were obtained from patients who had severe disease, and two were obtained from patients with mild disease (Table 1). Four of eight isolates produced stromal disease above the mean incidence of 34%. Two of these isolates came from patients who had severe disease, and two came from patients who had mild disease.

The incidence of stromal disease was at least 56% of the incidence of epithelial disease for all isolates except isolate 41, which produced a low incidence of stromal disease (25%) relative to the incidence of epithelial disease (70%).

Isolates 41 and 14 were from two patients who had bilateral HSV ocular disease. Both of these isolates produced epithelial disease in 70% of the mice inoculated. However, these isolates differed in that isolate 14 produced the highest incidence of stromal disease (60%), while isolate 41 produced a relatively low incidence of stromal disease (25%, Fig. 1).

Isolate 22 produced a very low incidence and mild severity of disease in the animal model. Only 1 of 20 mice had epithelial disease, and the same proportion developed stromal keratitis. The disease seen in the patient from whom the virus was isolated, however, was severe (Table 1). There was a history of 19 recurrences of disease beginning at age 6 yr in this patient. Six episodes of recurrent epithelial keratitis and episodes of stromal keratitis were observed in this patient by one of the authors (RDS). She required two penetrating keratoplasties in her affected eye (her first graft failed because of stromal herpes) and developed HSV epithelial keratitis postoperatively in both grafts.

Isolate 21 produced the highest incidence of epithelial disease (85%) and the second highest incidence of stromal disease (50%) in the animal model. In its human host, this isolate created a relatively mild pattern of disease. The patient had only two episodes of herpetic ocular disease separated by 7 yr. His first episode occurred at the age of 33 yr. As of the writing of this paper, he had been followed for 2.5 yr since his last episode. Both episodes were epithelial, without stromal involvement. Final visual acuity was 20/40, and penetrating keratoplasty was not required.

We compared the disease produced in mice by the two groups of viruses: (1) those that caused mild disease in their human hosts and (2) those that caused severe disease. There were no significant differences between the two groups. The incidence of dendrites was 44 for the mild group and 43 for the severe group,
and the incidence of stromal keratitis was 27 of 80 in each group. Similarly, there were no differences in the severity of dendritic disease ($P = 0.60$, by Wilcoxon rank-sum test) or in the severity of stromal keratitis ($P = 0.94$, by Wilcoxon rank-sum test). The power of our study was 80% to detect a 22% difference in the incidence of both epithelial and stromal disease between the two groups.

When it became apparent that there was no correlation between the severity of disease in human and murine hosts based on our initial criteria, we reviewed the charts to determine dendrite size, severity of initial attack, age at onset of first attack, and previous treatment with antiviral agents. Again, no correlation between these variables and the severity of disease in the mouse could be found.

**Discussion**

The eight different HSV isolates that we studied caused different patterns of ocular disease in the mouse, confirming previous reports of virally determined variation in the pattern of HSV ocular disease in animal models. However, the severity of disease in humans correlated poorly with the severity of disease in naive BALB/c mice after topical inoculation with the same virus isolates. There are several possible explanations for these data.

First, immunologic mechanisms are known to play a role in the pathogenesis of HSV stromal keratitis. Inbred athymic (nude) mice do not develop necrotizing stromal keratitis because they lack functional T-lymphocytes. Depletion studies implicate CD4+ (helper-inducer) T-cells in the pathogenesis of stromal keratitis. CD8+ cells also play an essential role in controlling the severity of stromal keratitis. Other studies suggest that resistance to stromal keratitis may be controlled by genes coding for immunoglobulin or closely linked to those loci.

Second, nonimmunologic host factors might also control the rate of spread and severity of HSV infections. We and others have found that structural cells from susceptible mouse strains support HSV replication better than cells from resistant strains. In contrast, fibroblasts from inbred mice differing in susceptibility to HSV infection by the intraperitoneal route were found in other laboratories to support the replication of HSV equally well in vitro, and inbred mice differing in susceptibility to central nervous system infection after footpad infection showed no difference in the ability of their ganglia to support the replication of HSV in vitro.
Third, viral factors that are important in determining the severity of disease in humans may be different from those that are important in the mouse. This would not be surprising because there are other aspects of HSV keratitis in the mouse model that differ from those in humans. For example, mice rarely, if ever, have spontaneous recrudescences of HSV keratitis; they are common in humans. Deaths from presumed HSV encephalitis and/or disseminated HSV infection are seen routinely in the mouse model, although they are rare in immunocompetent adult humans.

Fourth, the model of acute HSV ocular disease after topical corneal inoculation in naive inbred mice may not mimic the pattern of severe HSV keratitis typically seen in humans. For example, patients with blinding keratitis usually have multiple recurrences and, presumably, some degree of immunity to the virus. By contrast, the mouse model we studied focuses on a single primary infection in nonimmunized animals. Future experiments should examine possible correlations between disease severity in humans and latency or induced recrudescence in the animal model.

Key words: herpes simplex, keratitis, mice, stromal keratitis, cornea

References