Ocular Herpes Simplex Virus Reactivation in Mice Latently Infected With Latency-Associated Transcript Mutants

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A mouse model for ocular reactivation of herpes simplex virus type 1 (HSV-1) was modified and used to study the effect of strain difference on the frequency of ocular HSV reactivation. Outbred male NIH white mice were immunized with 1.0 ml of anti-HSV serum with a neutralizing titer of 1:400 24 hr before infection and bilaterally infected at 10⁵ plaque-forming units/eye with one of three HSV-1 strains: 17 Syn⁺, LAT⁺ (XC-20), or LAT⁻ (X10-13). Latency-associated transcripts (LAT) are produced by strain 17 Syn⁺ and LAT⁺ but not by LAT⁻. The primary infection was monitored by ocular swabbing for HSV. Reactivation was induced by intravenous (IV) injection of cyclophosphamide (5 mg) followed 24 hr later by IV dexamethasone (0.2 mg). These drugs significantly reduced the white cell count between 0 and 6 days postadministration. The eyes were swabbed for 7 consecutive days to monitor reactivation, and HSV-1 reactivation was induced at the following frequencies in individual eyes: 17 Syn⁺ (32.5%), LAT⁺ (18.5%), and LAT⁻ (2.5%) (P < 0.002). Co-culture of trigeminal ganglia was done, and random isolates were checked to ascertain their identity. The HSV was recovered from individual trigeminal ganglia at the following frequencies: 17 Syn⁺ (83%), LAT⁺ (100%), and LAT⁻ (67%) (P < 0.091). These results confirm that the mouse can be used as a reactivation model for ocular HSV infection and that the presence of LAT facilitates reactivation in vivo in the mouse. Invest Ophthalmol Vis Sci 32:1558-1561, 1991

A model for recurrent herpetic eye disease in mice was recently described. In this system, mice were passively immunized with serum containing antibodies to herpes simplex virus type 1 (HSV-1) 24 hr before inoculation of the cornea. Immunization reduced the incidence of severe eye damage and increased the rate of recovery of HSV after reactivation compared with a previous mouse model system reported by the same authors. Passive immunization protects the mouse eye by restricting the spread of HSV in the nervous system. Both mouse reactivation models induced HSV reactivation with ultraviolet B radiation plus intravenous cyclophosphamide and dexamethasone. Immunosuppression has also been used to induce reactivation in the mouse and rabbit, but the precise mechanism of reactivation is unknown.

A recent report suggests that HSV latent-phase transcription facilitates in vivo reactivation in the rabbit. Latency-associated transcripts (LAT) are the only known region of the HSV-1 genome to be transcribed in latently infected sensory neurons. However, the precise role of LAT is unclear, and several groups have ascertained that HSV mutants with deletions in LAT are capable of maintaining latent infections. This study was designed to determine whether LAT is an important component of in vivo reactivation in the mouse model of recurrent herpetic eye disease.

Materials and Methods

NIH Swiss Webster male mice were obtained from Sprague-Dawley (Indianapolis, IN). All animals were used at 6 weeks of age. All animals were cared for in accordance with the ARVO Resolution on the Use of Animals in Research.
The HSV-1 strains used were: (1) 17 Syn+; (2) LAT− (X10-13);5 and (3) LAT+ (XC-20),6 a strain derived by marker rescue of LAT− (X10-13). The LAT− (X10-13) represents an HSV-1 strain 17 Syn+ X HSV-II strain RS6 recombinant possessing a 1200-base pair deletion encompassing the promoter and 5' portion of HSV DNA encoding for LAT. The LAT+ (XC-20) was derived by co-transfection of genomic length DNA from X10-13 and a cloned Eco RI J and K (0.00-0.08 and 0.83-0.84 μm) fragment from HSV-1 strain 17 Syn+.

The mice were anesthetized and their corneas scarified with two horizontal and two vertical incisions. The corneas were inoculated with 5 μl of virus containing 10^5 plaque-forming units. The lids were closed and the eyes massaged for 30 sec.

The tear film was monitored for the presence of HSV during the acute infection (postinfection days 5 and 7) and after reactivation by wiping a small ocular swab over the cornea and around the fornices. The swab was placed in a tube with primary rabbit kidney indicator cells. The virus was allowed to elute for 30 min before removal of the swab. The indicator cells were monitored thereafter for cytopathic effect (CPE).

Serum raised against HSV was prepared by injecting rabbits with the virus. Serum from the rabbits was titrated in a standard manner. The mice received 1.0 ml of antiserum (neutralizing titer 1:400) raised against HSV by intraperitoneal injection 24 hr before corneal inoculation. Reactivation of latent HSV infections was induced by intravenous (IV) injection in the tail vein of 0.2 mg dexamethasone in phosphate-buffered saline (PBS) and 24 hr later by IV injection of 0.57 ml of phosphate-buffered saline (PBS) and 24 hr later by IV injection in the tail vein of 0.2 mg dexamethasone in 0.2 ml PBS.

Trigeminal ganglia were removed by sterile technique and placed in medium (Eagle’s Minimum Essential Medium with 10% fetal bovine serum and 200 mM dimethyl sulfoxide). Medium was removed from the organ culture every other day and placed on indicator cell monolayers. The cell monolayers were observed for CPE. The frequencies of in vitro reactivation, in terms of numbers of animals and numbers of eyes, and in vitro reactivation from trigeminal ganglia were analyzed by a chi-square test.14

### Table 1. In vivo HSV shedding after cyclophosphamide and dexamethasone immunosuppression

<table>
<thead>
<tr>
<th></th>
<th>17 Syn+ (+/total)</th>
<th>LAT+ (+/total)</th>
<th>LAT− (+/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>9/20 (45.0%)</td>
<td>6/19 (31.6%)</td>
<td>1/20 (5.0%)</td>
</tr>
<tr>
<td>Eyes</td>
<td>13/40 (32.5%)</td>
<td>7/38 (18.5%)</td>
<td>1/40 (2.5%)</td>
</tr>
</tbody>
</table>

### Table 2. In vitro HSV detected after organ culture of trigeminal ganglia

<table>
<thead>
<tr>
<th></th>
<th>17 Syn+ (+/total)</th>
<th>LAT+ (+/total)</th>
<th>LAT− (+/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>5/6 (83%)</td>
<td>6/6 (100%)</td>
<td>5/6 (83%)</td>
</tr>
<tr>
<td>Ganglia</td>
<td>10/12 (83%)</td>
<td>12/12 (100%)</td>
<td>8/12 (67%)</td>
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Blood was obtained from the tail vein of mice on four occasions: day 0, before administration of cyclophosphamide; day 2, after the administration of cyclophosphamide and dexamethasone; and days 4 and 6. Blood was similarly obtained from a control group of mice given IV PBS instead of cyclophosphamide and dexamethasone. Diluted samples were placed in a hemocytometer, and white blood cells were counted.

### Results

During the acute infection, 76%, 70%, and 71% of eyes infected with HSV strains 17 Syn+, LAT+, and LAT−, respectively, shed HSV detected by eye swabbing on at least one of the 2 days. Cyclophosphamide and dexamethasone in combination reduced the white cell count from approximately 4 X 10^6 to 1 X 10^4 on days 2 and 4 after receiving the drugs. By day 6, the white blood cell count had returned to the pre-treatment level and was similar to the control group.

Reactivation after immunosuppression occurred at the following frequencies: HSV-1 strain 17 Syn+, 45.0%; LAT+, 31.6%; and LAT−, 5.0%. The number of animals in each group was 19 or 20 (Table 1). The frequencies of reactivation after immunosuppression in individual eyes were: HSV-1 strain 17 Syn+, 32.5%; LAT+, 18.5%; and LAT−, 2.5%. The rates of in vivo reactivation for the three HSV-1 strains were significantly different, on either a per mouse or per eye basis (Table 1).

The trigeminal ganglia of six randomly selected mice from each group were assessed for latent virus by organ culture. Virus was recovered at the following frequencies (per animal): 17 Syn+, 83%; LAT+, 100%; and LAT−, 83% (Table 2). When in vitro reactivation from organ culture of individual ganglia was considered, the reactivation frequencies were: 17 Syn+, 83%; LAT+, 100%; and LAT−, 67%. There was no significant difference between these rates of reactivation on

### Table 3. Time of first detection of HSV after organ culture of trigeminal ganglia

<table>
<thead>
<tr>
<th></th>
<th>17 Syn+ (+/total)</th>
<th>LAT+ (+/total)</th>
<th>LAT− (+/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (days)</td>
<td>6–11</td>
<td>6–11</td>
<td>6–15</td>
</tr>
<tr>
<td>Mean (days)</td>
<td>7.2</td>
<td>9.4</td>
<td>9.4</td>
</tr>
</tbody>
</table>
either a per animal or per ganglion basis (Table 2). The virus was usually detected in the trigeminal ganglion after 6–8 days of organ culture. A slight delay in detection of the LAT− strain was apparent (Table 3). This difference was not statistically significant.

Discussion

During the primary infection, HSV was detectable by ocular swabbing from the tear film. Ocular swabbing is a quicker and less demanding method for sampling the tear film than ocular washing.1,2 Our HSV detection results during the primary infection (days 5 and 7) are similar to those reported by ocular washing on day 7, but less than those reported by ocular washing on days 3–6.2 A disadvantage of ocular swabbing is that the signs of HSV reactivation on the surface epithelium may be obliterated. (Recently, we started to exclude mice in which both eyes were negative for HSV during the acute infection. We find that after organ culture only 25% of ganglia from these animals have detectable HSV during the latent phase. This suggests that virus is unavailable for reactivation in the remaining 75% of ganglia. Unpublished results.)

Dexamethasone and cyclophosphamide in combination reduced the white cell count 0–6 days postadministration. No agent that has been associated with direct ocular trauma was used in our system, ie, ultraviolet B11 or epinephrine iontophoresis.15 The precise role of immunosuppression in inducing reactivation is uncertain. Possible factors include the suppression of cell-mediated immunity, direct toxic effects of cyclophosphamide on ganglion cells, or interruption of the normal mechanisms of DNA synthesis and repair.16

This study provided further evidence that LAT plays little or no role in the acute phase of viral infection or in the establishment and maintenance of latent HSV infections,6 because similar numbers of animals infected with each virus strain had HSV detectable in the tear film during acute infections and similar numbers of animals had HSV detectable by organ culture of the trigeminal ganglia. Since ocular titers of three viruses were not determined during the acute corneal epithelial infection in the mice, we cannot be certain that LAT plays no role in the acute infection in this model.

It does appear to facilitate in vivo reactivation in mice. These results are similar to those reported in the rabbit model.6 In the mouse system, reactivation induced by cyclophosphamide and dexamethasone produced reactivation frequencies of 31% and 5% of mice infected with LAT+ and LAT− strains, respectively. These results were not as high as the reactivation frequencies of 90% and 12% with identical LAT+ and LAT− mutants when epinephrine was used to induce in vivo reactivation of HSV in the rabbit.6 At present, the rabbit epinephrine iontophoresis model for HSV reactivation is a more efficient model to study reactivation. Leib et al11 reported that a deletion mutant of HSV-1 with a lesion in the promoter region for LAT had a reduced frequency of reactivation from explant culture. The LAT− mutant in our study reactivated from organ culture at a frequency similar to those of the LAT+ and 17 Syn+ strains (Table 2). Steiner et al10 also reported another HSV-1 deletion mutant in the region of LAT that significantly delayed the time of reactivation from explant culture in vitro. Again, the LAT− mutant in our study does not appear to have significantly delayed reactivation from explant culture in vitro (Table 3). However, the LAT− mutants used by other investigators9–13 were from HSV-1 strains and our mutant, X10-13, was an HSV-1/HSV-2 intertypic recombinant. These observations in mice compare with the results of similar explant culture experiments using these deletion mutants in the rabbit.6 Variations in the precise location of the dysfunctional region of LAT may account for variations reported in in vitro explant culture studies with different LAT mutants. Taken together, the results of this study suggest that there may be a difference in the mechanisms of in vivo and in vitro HSV reactivation.

Key words: HSV, LAT, latency, mice, ocular, reactivation

References


