Efficacy of Herpes Simplex Virus Type 1 Immunization in Protecting Against Acute and Latent Infection by Herpes Simplex Virus Type 2 in Mice

ROBERT R. McKENDALL

Department of Neurology, Veterans Administration Hospital and University of California, San Francisco, California 94121

Received for publication 22 February 1977

ICR mice were immunized with herpes simplex virus type 1 (HSV-1) and later challenged with HSV-2 by footpad inoculation. Both immunized animals and age-matched, nonimmunized controls were observed for ascending neurological disease and latent infection of spinal ganglia resulting from the HSV-2 challenge. Control animals had a 78% incidence of acute and latent infection compared with a 1.7% incidence in immunized mice. The data show immunity to HSV-1 is protective against both acute and latent infection by HSV-2.

Increasing evidence has accumulated over the last few years to document the existence of two closely related subtypes of herpes simplex virus (HSV) — HSV-1 and HSV-2 (9-11, 14). Peripheral inoculation of either subtype into mice and other experimental animals results in a neurological disease characterized by progressive paralysis of the limb of the inoculated side and later of the opposite side (15-18). Various studies show that the occurrence of ascending neurological disease is accomplished by neural transit of virus (1-4, 21).

A variety of immunological studies suggest that host factors may moderate both the appearance and severity of disease (5, 7, 8, 12, 13, 19). Although knowledge of cross-neutralizing antibody in vitro (i.e., antibody to one HSV subtype neutralizes the other subtype) has been available for several years, investigation of in vivo cross-immunity has not until now been undertaken.

Epidemiological data in man associate HSV-1 with supradiaphragmatic sites of infection whereas HSV-2 primarily occurs about genitally related areas (9). Up to 55% of people develop neutralizing HSV-1 antibody prior to the onset of sexual activity (20). In light of recent increases of all human venereal diseases, including HSV-2, a study was undertaken in mice to determine whether prior HSV-1 exposure and immunity was protective against the ascent of HSV-2 along nerves. This design was chosen not only because HSV-2 exposure presumably occurs after attainment of sexual maturity, and hence later than HSV-1 in most people, but also because HSV-2 is more neurovirulent (16) and, therefore, might circumvent the immune system ability to clear virus.

Immunization of 4-week-old ICR mice was accomplished by footpad inoculation of a single dose containing 1,300 50% tissue culture infective doses (TCID_{50}) of HSV-1, a human strain previously described (6). Three weeks later, the HSV-1-immunized mice and 55 age-sex-matched control mice, which had been held concurrently in our animal facility, were inoculated in the opposite footpad with 1,000 TCID_{50} of HSV-2, a strain isolated from a human penile vesicle. Animal groups were coded and evaluated every day or two for evidence of ascending neurological disease. After the period of acute illness was completed, the code was broken and the results were tabulated. Following a minimum interval of 12 weeks after HSV-2 challenge, study of spinal ganglia for the presence of latent infection was done by explant technique. The lumbosacral dorsal root ganglia from both sides of the illness-free animals were aseptically removed and cultured in petri dishes with medium 199 supplemented with antibiotics, 1% glutamine, and 10% heat-inactivated fetal calf serum. Culture supernatants were taken at 1- to 5-day intervals over the course of 30 days and frozen for later virus assay by observation of a cytopathic effect on primary rabbit kidney cell culture. All isolates were identified by complete neutralization with HSV hyperimmune guinea pig serum. HSV typing by neutralization kinetics (17) was kindly provided by Ron Duff (North Chicago, Ill.).

Following immunization with HSV-1, 92 of 250 (36.8%) mice developed neurological disease. Of these mice, 63 recovered monoplegic animals and 58 mice that had remained well were challenged with HSV-2. Table 1 shows
that neurological illness resulting from HSV-2 challenge developed in 36 to 53% of the control mice compared with 0 to 4% of the mice immunized with HSV-1 (P < 0.001). Among the HSV-1-immunized mice, prior HSV-1 disease did not influence the response to HSV-2 challenge.

Since animals that remain free of illness may harbor latent virus in the dorsal root ganglia, study of illness-free animals after HSV-2 challenge for possible latent ganglionic infection was undertaken to determine whether the ascent of HSV-2 along nerves had been completely averted, or whether illness alone had been prevented by the HSV-1 immunization. Of 45 immunized mice which remained illness-free upon HSV-2 challenge, 23 had latent virus only on the side of HSV-1 immunization, and three had latent infection on both sides (Table 2). To determine whether latent infection on the side of HSV-2 challenge was the result of the HSV-1 immunization or of the HSV-2 challenge, isolates from these animals were typed and identified as HSV-1 by neutralization kinetics. In fact, all 45 mice were protected against ascent of HSV-2. In comparison, 18 illness-free control animals were studied, and six (33%) were shown to harbor latent virus on the side of the HSV-2 challenge. Therefore, the HSV-1-immune animals had a clearly lower incidence of latent HSV-2 infection compared with nonimmunized controls (P < 0.05).

To assess the overall effectiveness of HSV-1 immunization on the prevention of HSV-2 ascent along peripheral nerves, the prevention of combined acute and latent infection must be considered. Table 3 indicates that 1.7% of the HSV-1-immunized mice developed acute or latent infection compared with 78% of the unimmunized mice (P < 0.001).

The mechanism of this cross-immunity is unclear. Although the titer of neutralizing antibody in pooled sera from six immunized mice was 1:16 1 day prior to HSV-2 challenge, further studies will be required to determine whether humoral or cell-mediated immunity provides the protection. The data indicate that HSV-1 immunity under some circumstances may prevent the ascent of HSV-2 along nerves, resulting in aversion of both acute and latent herpetic infection. Consideration should be given to the concept that prior HSV-1 exposure may be an important influence on the acquisition of acute and latent HSV-2 disease in humans.

LITERATURE CITED


---

### Table 1. Ascending neurological disease in mice challenged with HSV-2 after immunization with HSV-1

<table>
<thead>
<tr>
<th>Group</th>
<th>Illness after HSV-2 challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt 1</td>
</tr>
<tr>
<td>HSV-1 immune</td>
<td>0/69*</td>
</tr>
<tr>
<td>Nonimmune controls</td>
<td>9/25</td>
</tr>
</tbody>
</table>

* Number sick/number of mice inoculated with 1,000 TCID<sub>50</sub> of HSV-2 by footpad.

### Table 2. Asymptomatic latent ganglionic infection in immunized versus nonimmunized mice challenged with HSV-2

<table>
<thead>
<tr>
<th>Group</th>
<th>Right side (immunized with HSV-1)</th>
<th>Left side (challenged with HSV-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive/no. tested</td>
<td>Isolate subtype</td>
</tr>
<tr>
<td>HSV-1 immunized Controls</td>
<td>26/45*HSV-1</td>
<td>3/45 HSV-1</td>
</tr>
</tbody>
</table>

* Number of mice with latent infection by explant technique/number of mice explanted.

### Table 3. Effect of HSV-1 immunization on the ascent of HSV-2 along nerves in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Acute HSV-2 disease</th>
<th>Latent HSV-2 infection in illness-free mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% No.</td>
<td>% No.</td>
</tr>
<tr>
<td>HSV-1 immune</td>
<td>1.7</td>
<td>121</td>
</tr>
<tr>
<td>Nonimmune controls</td>
<td>45</td>
<td>55</td>
</tr>
</tbody>
</table>

---