Pathogenesis of Zosteriform Spread of Herpes Simplex Virus in the Mouse

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SUMMARY

Zosteriform spread of herpes simplex virus (HSV) infection occurs after primary inoculation of the skin of both outbred and inbred mice. With HSV type I strain SC16 few outbred animals died if they were inoculated when 8 weeks old whereas up to 50% of animals died if inoculated when 4 weeks old. However, at either age, zosteriform spread of infection occurred in almost all animals as it did when 4-week-old outbred animals were inoculated with the avirulent strain KOS. Thus, control of zosteriform spread must act by different mechanisms from those controlling the encephalitis which leads to death. During replication in the epidermis virus enters axons and could first be found in sensory ganglia 2 days after inoculation of the skin. Thereafter, it was found in the nerve roots and in skin within the same dermatome but remote from the site of inoculation. When sensory nerves to this latter area were cut during the 4 days after primary inoculation lesions developing as a result of zosteriform spread were either completely inhibited or, with later section, decreased in incidence. Mortality was not affected by such nerve section. Latent infection must be established in neurons serving areas of skin remote from the inoculation site since with HSV-1 strain SC16, recrudescent lesions on the pinna could be induced by stripping the skin of the ear when the original inoculation had been in the skin of the neck. Such recrudescent disease was not demonstrated in animals infected with HSV-1 strain KOS even though this virus efficiently established latent infection in sensory ganglia.

INTRODUCTION

Since the studies of Goodpasture & Teague (1923) and Goodpasture (1929) it has been observed frequently that herpes simplex virus (HSV) can spread centripetally via peripheral nerves from a peripheral inoculation site to the central nervous system (CNS) (for review, see Baringer, 1975). Further experiments by Teague & Goodpasture (1923) first demonstrated that zosteriform spread of lesions (limited by dermatomal boundaries) could occur in rabbits and guinea-pigs after inoculation of HSV into a small area of a dermatome on the flank. They hypothesized that such lesions were caused by centrifugal flow of virus down peripheral nerves to the skin. Zosteriform spread of lesions caused by HSV was subsequently described in other experimental animals (for review, see Hill, 1983). However, there have been few detailed virological and pathological studies of the phenomenon (Constantine et al., 1971; Dillard et al., 1972) and Teague and Goodpasture’s hypothesis has never been fully tested. We now describe observations on the pathogenesis of zosteriform spread of HSV in the mouse using a development of the mouse ear model (Hill et al., 1975).

METHODS

Viruses. HSV-1 strain SC16, HSV-2 strain AR11 (Hill et al., 1975) and HSV-1 strain KOS (kindly supplied by Dr B. Roizman, Chicago, Ill., U.S.A.) were used.

Mice. All mice used were female. MF1-Ola hairless and NIH-Ola (inbred) were purchased from Olac, 1976 Ltd., Bicester, Oxon, U.K.

Previously, most of the work reported from this laboratory was done with animals from a closed colony of outbred Swiss white mice maintained in this Department. An outbreak of Sendai virus infection made it necessary
Inoculation of mice. Three methods were used; in each case mice were anaesthetized by intraperitoneal (i.p.) injection of sodium pentobarbitone: (i) subcutaneous (s.c.) injection of 20 μl of a suspension of virus in maintenance medium into the right ear (Hill et al., 1975); (ii) scarification of the upper surface of the right pinna through a 10 μl drop of virus suspension (ten parallel scarifications were made with a 26 gauge hypodermic needle); (iii) scarification of the right side of the ventral surface of the neck through a 10 μl drop of virus suspension. The neck was shaved and a rectangular box (5 mm side along the midline, 7 mm side towards the right ear) was drawn on the skin (Fig. 1a). The virus suspension was spread within the marked rectangle and this area was scarified in a manner similar to scarification of the ear. The 10 scarifications were parallel to the cranio-caudal axis.

Observation of lesions. Mice were anaesthetized and examined by ×15 magnification with a dissecting microscope.

Isolation of virus during acute infection. Mice were killed by i.p. injection of sodium pentobarbitone and tissues were ground immediately. The rectangle of skin at the inoculation site was removed and ground in 0.5 ml of maintenance medium. The skin was scraped from the dorsal surface of the right ear and ground in 0.5 ml of medium. The dorsal root entry zones of the second and third cervical nerves (including about 1 mm into the spinal cord) were removed, followed by the ganglia themselves. Each ganglion and root was ground separately in 0.2 ml of maintenance medium. From each tissue two 50 μl samples and serial 10-fold dilutions were inoculated separately on to Vero cells grown in Multi dishes (Sterilin) for assay of p.f.u. (Harbour et al., 1983).

Demonstration of latent infection in ganglia. Latent infection of the right second and third cervical ganglia was demonstrated by the method described previously (Harbour et al., 1983).

Induction of recurrent disease. The upper surface of the right ear was stripped with cellophane tape (Hill et al., 1978). Recurrent disease was assessed by microscopical observation of the development of herpetic lesions (Hill et al., 1982).

RESULTS

Demonstration of zosteriform spread of lesions

In preliminary experiments 4-week-old hairless mice were inoculated s.c. into the skin of the right flank near to the dorsal midline with 20 μl containing 10^5 or 10^6 p.f.u. of HSV-1 strain SC16. Four to 6 days later vesicular herpetic lesions developed in a band on the inoculated side extending from the inoculation site to the ventral midline (Hill, 1983). The number of lesions varied from a few scattered ones to a confluent band extending over the whole dermatome. Of 19 mice inoculated with 10^5 p.f.u. SC16, two developed such zosteriform spread; when 10^6 p.f.u. was used, 14 of 20 (70%) mice developed such spread, and six of these died, together with two which did not show lesions.

Delineation of the dermatome including the pinna of the ear

In order to delineate the dermatome that includes the ear pinna, groups of 5-week-old hairless mice were inoculated s.c. with 5 × 10^5 p.f.u. HSV-1 strain SC16 in 10 μl at the following sites on and around the head: just above the right eye, into the right side of the lower lip, just behind the right ear on the top of the head, in the skin of the neck just below the right ear, or into the skin of the right pinna. The pattern of zosteriform spread was used to make a map of the dermatomes of the head and neck. One dermatome included an area from the midline on top of the head to the base of the pinna, the pinna itself and a band of skin from the root of the ear to the midline on the ventral surface of the neck (Fig. 1b, c). Dissection revealed the dermatome to be innervated by nerves arising from the second and third cervical ganglia. An area on the ventral surface of the neck within this dermatome (Fig. 1a) was chosen as an inoculation site suitably distant from the ear for experiments on zosteriform spread to the pinna.

Zosteriform spread of lesions to the pinna

Groups of 4-week-old Bristol/2 mice were inoculated by scarification on the neck with 10^4 or 10^5 p.f.u. HSV-1 strain SC16. Animals were examined daily for the development of erythema and lesions on the right pinna and at the inoculation site. With a dose of 10^5 p.f.u. the skin at the
Zosteriform spread of HSV

Table 1. Deaths and development of lesions by zosteriform spread after inoculation with different strains of HSV in outbred and inbred mice

<table>
<thead>
<tr>
<th>Mouse* strain</th>
<th>Age (weeks)</th>
<th>Virus strain</th>
<th>Dose (p.f.u.) t</th>
<th>Deaths/total (%)</th>
<th>Lesions on ipsilateral ear/survivors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bristol/2 outbred</td>
<td>4</td>
<td>HSV-1 KOS</td>
<td>$10^5$</td>
<td>0/24 (0)</td>
<td>15/24 (63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^6$</td>
<td>0/22 (0)</td>
<td>19/22 (86)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-1 SC16</td>
<td>$10^4$</td>
<td>6/72 (8)</td>
<td>7/66 (10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^5$</td>
<td>88/188 (47)</td>
<td>72/100 (72)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-2 AR11</td>
<td>$10^4$</td>
<td>10/21 (47)</td>
<td>2/11 (18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^5$</td>
<td>6/13 (46)</td>
<td>5/7 (71)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^6$</td>
<td>8/13 (62)</td>
<td>3/5 (60)</td>
<td></td>
</tr>
<tr>
<td>Bristol/2 outbred</td>
<td>8</td>
<td>HSV-1 SC16</td>
<td>$10^5$</td>
<td>5/62 (8)</td>
<td>40/57 (70)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-2 AR11</td>
<td>$10^5$</td>
<td>1/29 (3.5)</td>
<td>17/28 (61)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^6$</td>
<td>0/26 (0)</td>
<td>12/26 (46)</td>
<td></td>
</tr>
<tr>
<td>NIH inbred</td>
<td>4</td>
<td>HSV-1 SC16</td>
<td>$10^5$</td>
<td>2/41 (5)</td>
<td>34/34 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^6$</td>
<td>0/28 (0)</td>
<td>28/28 (100)</td>
<td></td>
</tr>
</tbody>
</table>

* All mice were female.
† Inoculation by scarification on right side of neck.
‡ Five mice died accidentally.

inoculation site became swollen in most animals 2 to 3 days after inoculation. This swelling quickly resolved but was usually followed on day 5 or 6 by extensive scabbed lesions.

Erythema of the pinna was first seen on the 4th day after inoculation and reached a peak incidence of about 95% on days 5 and 6. In about 90% of animals the erythema was followed, usually within 24 h, by the appearance of herpetic vesicles on the ear. These lesions were always in erythematous skin and quickly progressed to pustules and crusted lesions. Within 4 days of their emergence the lesions usually disappeared. With scarification through $1 \times 10^5$ p.f.u., mortality and the incidence of zosteriform spread were considerably greater than with $1 \times 10^4$ p.f.u. (Table 1). The number of lesions varied from 1 to 50.

When 8-week-old Bristol/2 mice were inoculated similarly with $10^5$ or $10^6$ p.f.u. only about 5% of animals died but the incidence of zosteriform spread to the ear was 73% and 91% respectively. However, many of these mice which had shown zosteriform spread subsequently
suffered extensive damage to the pinna probably as a result of continued scratching and were unsuitable for experiments on recurrent disease.

Mice of the NIH strain were also tested. Of 69 4-week-old animals inoculated in the neck with 10^5 or 10^6 p.f.u. HSV-1 strain SC16 very few died and all survivors developed lesions on the pinna. In further experiments 4-week-old Bristol/2 mice were inoculated in the neck with 10^4 to 10^7 p.f.u. HSV-1 strain KOS. No mice died at any dose although up to 86% developed lesions on the ear. Infection of 4-week-old Bristol/2 mice with 10^4 to 10^6 p.f.u. of HSV-2 strain AR11 resulted in up to 62% mortality. However, mortality was negligible when 8-week-old mice were similarly infected even though up to 61% of mice showed zosteriform spread of lesions to the pinna (Table 1).

The gross morphology of lesions that developed on the pinna was independent of strain or age of mice and strain of virus and was identical to that of recurrent lesions induced in the pinna by stripping with cellophane tape (Hill et al., 1982). Microscopical examination showed that productively infected cells were limited to the epidermis. The earliest vesicular lesion was extensively infiltrated in the dermis and epidermis with polymorphonuclear leukocytes.

**Spread of infection through tissues**

Four-week-old Bristol/2 mice were inoculated by scarification in the skin of the neck through 10^5 p.f.u. HSV-1 strain SC16. Skin from the inoculation site and from the ipsilateral ear, together with the ipsilateral cervical ganglia 2 and 3 and their nerve roots were taken daily for 9 days from 12 animals on each occasion. In addition, skin from the inoculation site was taken 3 h after inoculation.

HSV was isolated from the inoculation site of virtually all animals up to the 6th day after inoculation. Peak titres (about 2 x 10^5 p.f.u./sample) occurred on the 2nd day; thereafter, the amount of virus slowly declined. Virus was not isolated from ganglia 1 day after inoculation but on the 2nd day seven of the 12 second cervical ganglia yielded virus. Thereafter, virus was isolated from virtually all ganglia up to day 6. Peak titres (about 10^3 p.f.u./ganglion) occurred on day 4 but there was little change in the titre in the period day 3 to day 6. Similar results were obtained from the third cervical ganglion. Isolation from the second cervical nerve root lagged 1 day behind that from the ganglion with only one isolation on the 2nd day after inoculation and eight on the 3rd day. Thereafter, until the 6th almost all nerve roots yielded virus with peak titres of about 7 x 10^2 p.f.u. on day 6. Results from the third cervical nerve root were similar. Virus was first isolated from the skin of the ear (from three of 12 animals) 3 days after inoculation. Thereafter, until day 7 almost all samples of ear skin yielded virus with peak titres of about 2 x 10^6 p.f.u./sample on the 5th and 6th day.

The majority of samples from skin at the inoculation site, ganglia and nerve roots failed to yield virus 7 days after inoculation and on the 8th and 9th days isolations were only sporadic. By contrast, 11 of the 12 samples of ear skin yielded virus on the 7th day, and on the 8th day there were six isolations from this site (Table 2). The fact that infection with HSV-1 strain KOS did not kill mice although zosteriform spread of lesions (and latent infection in ganglia, see below) was common, prompted investigation of the spread of this infection. Titres of virus from cervical ganglia and their nerve roots and the incidence and timing of the isolations (Table 3) were indistinguishable from the results of similar tests after inoculation with HSV-1 strain SC16.

**Section of nerves to the pinna during primary infection**

Four-week-old Bristol/2 mice were inoculated by scarification in the skin of the neck through 1 x 10^5 p.f.u. SC16. Daily from the 1st to the 4th day after inoculation groups of mice were selected at random and the main sensory nerves (greater auricular and lesser occipital) from cervical ganglia 2 and 3 to the pinna were cut close to the base of the pinna. The branches of the facial nerve to the ear (the motor nerve to the muscles of the pinna) and branches of the cervical nerves to the skin of the neck were avoided. Surgery for control mice was similar except that the nerves were left intact. On the 1st and 3rd days after inoculation other control groups were
Table 2. Isolation of HSV from inoculation site (neck), cervical ganglia, nerve roots and ipsilateral ear during primary infection

<table>
<thead>
<tr>
<th>Time after inoculation (days)</th>
<th>Skin of inoculation site</th>
<th>Cervical ganglia</th>
<th>Nerve roots</th>
<th>Skin of ear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C2</td>
<td>C3</td>
<td>C2</td>
</tr>
<tr>
<td>0*</td>
<td>1-8 ± 0-3‡</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>3-6 ± 0-8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5-3 ± 0-4</td>
<td>7</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>4-7 ± 1-0</td>
<td>3-2 ± 0-6</td>
<td>2-8 ± 0-6</td>
<td>1-6 ± 1-1</td>
</tr>
<tr>
<td>4</td>
<td>5-0 ± 0-5</td>
<td>2-9 ± 0-9</td>
<td>2-9 ± 1-2</td>
<td>2-6 ± 0-9</td>
</tr>
<tr>
<td>5</td>
<td>4-4 ± 1-1</td>
<td>2-7 ± 1-0</td>
<td>2-6 ± 1-1</td>
<td>2-4 ± 0-9</td>
</tr>
<tr>
<td>6</td>
<td>4-1 ± 0-7</td>
<td>2-7 ± 0-7</td>
<td>2-5 ± 0-9</td>
<td>2-5 ± 1-0</td>
</tr>
<tr>
<td>7</td>
<td>3-2 ± 1-0</td>
<td>1-2 ± 1-0</td>
<td>0-8 ± 0-7</td>
<td>1-2 ± 0-7</td>
</tr>
<tr>
<td>8</td>
<td>3-4</td>
<td>2-1</td>
<td>1-6</td>
<td>1-1 ± 1-3</td>
</tr>
<tr>
<td>9</td>
<td>0-7</td>
<td>0</td>
<td>0-3</td>
<td>0-4 ± 0-2</td>
</tr>
</tbody>
</table>

* Three h after inoculation by scarification of neck.
† Number of samples yielding virus (12 were taken at all times except on days 8 and 9 when there were 11).
‡ log10 Geometric mean titre/sample yielding virus ± s.d.
§ ND, Not done.

Table 3. Yields of HSV from cervical ganglia 2 and 3 and nerve roots of mice* inoculated in the skin of the neck by scarification through 1 × 10^6 p.f.u. HSV-1 KOS

<table>
<thead>
<tr>
<th>Time after inoculation (days)</th>
<th>Cervical ganglia†</th>
<th>Nerve roots†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolation/total</td>
<td>Titre‡ ± S.D.</td>
</tr>
<tr>
<td>3</td>
<td>10/10</td>
<td>4-4 ± 0-6</td>
</tr>
<tr>
<td>4</td>
<td>10/10</td>
<td>4-2 ± 0-7</td>
</tr>
<tr>
<td>5</td>
<td>9/10</td>
<td>2-4 ± 0-8</td>
</tr>
</tbody>
</table>

* Mice were 4-week-old, female Bristol/2.
† The two ganglia from each mouse were pooled as were the two nerve roots (the root samples included a small amount of spinal cord).
‡ log10 Geometric mean p.f.u./specimen of those with virus.

merely anaesthetized and shaved. The effects of surgery were assessed by observing the incidence of death and herpetic lesions on the pinna. Treatments were coded.

Section of the nerves 1 day after inoculation completely prevented development of lesions on the pinna (Table 4). Delay of surgery allowed an increase of zosteriform spread of lesions to the ear, but even with nerve section on the 4th day after inoculation the incidence (58%) was lower than in control groups (where it was 73 to 100%). Mortality was not substantially altered by surgery at any time. Latent infection in cervical ganglia 2 and 3 was demonstrated in all 59 mice whose nerves were cut; in control groups the incidence was 64 of 68 (94%).

Incidence of latent HSV infection in cervical ganglia

The incidence of latent infection in cervical ganglia 2 and 3 was observed in mice that survived from experiments where such variables as dose and strain of virus, strain and age of mice, or route of inoculation had been assessed. Latent infection was demonstrable in virtually
Table 4. Effect of nerve section on death and zosteriform spread of HSV-1 infection to ear

<table>
<thead>
<tr>
<th>Surgery (days after inoculation)*</th>
<th>Lesions on ear/total</th>
<th>Deaths/total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nerve section‡ No./total (%)</td>
<td>Mock section No./total (%)</td>
</tr>
<tr>
<td>1</td>
<td>0/54 (0)</td>
<td>29/49 (73)</td>
</tr>
<tr>
<td>2</td>
<td>4/26 (15)</td>
<td>10/12 (83)</td>
</tr>
<tr>
<td>3</td>
<td>11/23 (48)</td>
<td>12/15 (80)</td>
</tr>
<tr>
<td>4</td>
<td>7/12 (58)</td>
<td>10/10 (100)</td>
</tr>
</tbody>
</table>

* Skin on right side of neck scarified through 10 µl drop containing 10⁵ p.f.u. HSV-1 SC16. Mice were 4-week-old, female Bristol/2.
† After shaving behind right ear the right lesser occipital and greater auricular nerves were cut.
‡ ND, Not done.

Table 5. Incidence of induced* recurrent disease and latent infection with HSV

<table>
<thead>
<tr>
<th>Method of inoculation</th>
<th>Site</th>
<th>Dose (p.f.u.)</th>
<th>Incidence of recurrent lesions No./total‡ (%)</th>
<th>Incidence of latent infection in cervical ganglia No./total‡ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous</td>
<td>Ear</td>
<td>10⁴</td>
<td>ND‡</td>
<td>4/25 (16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 × 10⁴-3 × 10⁵</td>
<td>13/84 (16)</td>
<td>34/85 (40)</td>
</tr>
<tr>
<td>Scarification</td>
<td>Ear</td>
<td>10⁴</td>
<td>9/40 (23)</td>
<td>30/44 (68)</td>
</tr>
<tr>
<td></td>
<td>Ear</td>
<td>10⁵</td>
<td>11/38 (29)</td>
<td>33/41 (81)</td>
</tr>
<tr>
<td></td>
<td>Neck</td>
<td>10⁴</td>
<td>4/59 (7)</td>
<td>7/56 (12)</td>
</tr>
<tr>
<td></td>
<td>Neck</td>
<td>10⁵</td>
<td>20/66 (30)</td>
<td>110/111 (99)</td>
</tr>
<tr>
<td></td>
<td>Neck</td>
<td>10⁵</td>
<td>12/39 (31)</td>
<td>74/75 (99)</td>
</tr>
</tbody>
</table>

(NIH inbred)§

* Induced by cellophane tape stripping of right ear.
† No. with lesions, or yielding virus from ganglia/no. tested.
‡ ND, Not done.
§ All other results were with Bristol/2 outbred mice; all were 4 weeks old when inoculated with HSV-1 SC16.

all Bristol/2 mice inoculated by scarification of the neck through at least 1 × 10⁵ p.f.u. HSV-1 strain SC16 (Table 5). Results were the same whether these animals were 4 weeks or 8 weeks old when inoculated. Four-week-old NIH mice inoculated in the same way responded similarly; 74 of 75 were latently infected. The inoculum dose had considerable effect on the incidence of latency. Thus only seven of 56 animals scarified through 1 × 10⁴ p.f.u. HSV-1 strain SC16 were shown to be latently infected. The route of inoculation also affected the incidence of latent infection. Scarification of the pinna of 4-week-old Bristol/2 mice through 1 × 10⁵ p.f.u. HSV-1 strain SC16 resulted in latent infection in 33 of 41 animals (81%); with 1 × 10⁴ p.f.u. 30 of 44 animals (68%) were latently infected. However, with 1 × 10⁵ to 3 × 10⁵ p.f.u. injected s.c. latency was demonstrated in only 34 of 85 (40%) animals and with a dose of 1 × 10⁴ p.f.u. the figure was four of 25 (16%).

Viruses of different pathogenicity were also tested. HSV-2 strain AR11 inoculated by scarification in the neck induced latent infection in all mice that survived doses of virus > 1 × 10⁵ p.f.u., in eight of 11 mice scarified through 1 × 10⁴ p.f.u., but in none of 15 where 1 ×
Zosteriform spread of HSV

10^3 p.f.u. was used. HSV-1 strain KOS also established latent infection efficiently, in all mice scarified through 1 × 10^6 or 1 × 10^7 p.f.u., in 21 of 23 mice with 1 × 10^5 and in 17 of 23 with 10^4 p.f.u.

Incidence of recurrent disease

Four to 14 weeks after inoculation the skin of the pinna on the inoculated side was stripped with cellophane tape to induce recurrent disease. Lesions developed on the pinna of about 30% of either NIH or outbred mice previously inoculated by scarification in the skin of the neck through 1 × 10^5 p.f.u. HSV-1 strain SC16 (Table 5), an incidence similar to that in outbred mice scarified in the skin of the pinna through the same dose of virus. When 1 × 10^5 p.f.u. had been used for similar inoculations 23% of animals showed recurrent disease after stripping the skin. When virus had been inoculated s.c. (outbred mice) the incidence of recurrent disease after stripping the skin was less (16%). Results from outbred animals inoculated by scarification with HSV-1 strain KOS were quite different. Of about 20 mice inoculated through doses of each of 10^4, 10^5, 10^6 and 10^7 p.f.u, none developed recurrent lesions after stripping the skin of the ear.

It was difficult to determine accurately the incidence of recurrent lesions in mice originally infected with HSV-2 strain AR11. Many of these animals scratched the pinna to such an extent that it was not possible to do the test of stripping with cellophane tape. In others the self-inflicted damage made interpretation of observation of the skin impossible. For this reason observations after stripping the ears of mice originally inoculated with 1 × 10^6 or 1 × 10^5 p.f.u. HSV-2 strain AR11 were abandoned. Seven animals originally inoculated with 1 × 10^4 p.f.u. and 16 with 1 × 10^3 p.f.u. were tested; none developed recurrent lesions. Again, because of self-inflicted damage, only small numbers of animals inoculated with HSV-1 strain SC16 when 8 weeks old could be tested. On the first occasion of stripping four of 17 animals developed recurrent lesions; on the second occasion (4 weeks later) lesions were seen in four of 14 animals. One animal developed lesions after both occasions of stripping.

The continuing inflammation in the skin of some animals infected with HSV-1 strain SC16 when 8 weeks old might have been due to persisting active infection. Therefore, 15 weeks after inoculation attempts were made to isolate infectious virus from cervical ganglia 2 and 3 and the pinna. Of eight mice with inflamed ears none yielded virus from either site; of 15 with normal ear skin, virus was isolated from skin once but never from ganglia.

DISCUSSION

The historic work by Teague & Goodpasture (1923) showed that, in guinea-pigs and rabbits, HSV could produce a distribution of clinical lesions that mimicked that of herpes zoster in humans, hence the term zosteriform spread of lesions. Induction of inflammation in the skin, for instance by the application of coal tar, was necessary to induce such lesions but these occurred only in the inoculated dermatome, and not throughout the area of inflammation. This effect of inflammation of the skin finds a parallel in our much later observations that in mice mild inflammation is a prerequisite for the production of recurrent herpes lesions (Hill et al., 1978; Harbour et al., 1983). Later sporadic reports have shown that HSV can establish zosteriform lesions in rats (Tanaka & Southam, 1965), mice (Sydiskis & Schultz, 1965; Constantine et al., 1971; Dillard et al., 1972; Robinson & Dover, 1972) and man (Music et al., 1971; Mok, 1971) without treating the skin with inflammatory agents. Zosteriform spread is also the likely explanation for the occurrence of lesions on the external genitalia of guinea-pigs after intravaginal inoculation of HSV-2 (Stanberry et al., 1982).

The present report establishes that in mice HSV infection regularly shows zosteriform distribution of lesions and that this must be regarded as a normal pattern of the primary infection after inoculation of the skin. Furthermore, the spread of infection through the skin results from a complex sequence of replication of virus within the nervous system. Invasion by the virus of the whole dermatome including the original site of inoculation may complicate and prolong primary infection at this site. Within 2 days of inoculation in the skin the virus is present in the sensory ganglia, a frequent finding now generally accepted to result from axonal transport of virus from the inoculated skin (for review, see Hill, 1984). Ample evidence also shows that
virus replicates in the neurons of the ganglia (Cook & Stevens, 1973; Dillard et al., 1972; Knotts et al., 1974). Since the nerve root samples contained mostly CNS tissue the virus probably invades the CNS via the nerve roots within 3 days of introduction into the skin. Indeed, within 5 days of inoculation all mice had virus in the nerve root samples. This result is similar to our previous reports using s.c. inoculation of the pinna (Hill et al., 1983a) or scarification of the cornea (Tullo et al., 1982a) as the route of inoculation.

In all animals, from 5 to 7 days after inoculation, virus was found in the skin of the pinna, i.e. a quite separate area from the site of inoculation in the neck. The restriction of lesions by dermatomal area indicates strongly that this spread is via nerves but the evidence from the experiments where nerves to the pinna were sectioned makes this conclusion inescapable. For such spread to occur the virus must escape from the neuron whose axon it originally invaded to others serving separate areas of skin. Possible routes for this spread have been discussed previously (Hill, 1983). Briefly, the three most likely are (i) axon to axon spread within the peripheral nerve, (ii) neuron cell body to cell body spread in the ganglion and (iii) neuron to neuron spread within the CNS (perhaps also involving glial cells). Physical barriers make routes (i) and (ii) unlikely or inefficient. By contrast, virus replicates freely in oligodendrocytes and astrocytes (Townsend & Baringer, 1976, 1978) which connect with many CNS neurons. Hence, route (iii) is considered the most likely to allow development of a zosteriform pattern of lesions and the CNS part of the root entry zone may be the site vital to such spread.

Section of nerves to the ear 1 day after inoculation of virus into the skin of the neck completely abrogated development of lesions in the skin of the ear but had no effect on the number of mice that died or on the incidence of latent infection in the 2nd and 3rd cervical ganglia. These observations emphasize that surgery did not interfere with access of virus to the ganglia and CNS via nerves from the inoculation site.

The finding that nerve section even 4 days after inoculation still decreased the incidence of lesions in the pinna (although to a diminishing extent with an increasing interval after inoculation) suggests that virus continues to pass centrifugally along these nerves for at least this time. This continued transport probably reflects continued replication within CNS neurons or glial cells.

Isolation of virus from the pinna of 25% of mice as early as 3 days after inoculation of the neck and the occurrence of lesions on the pinna in a similar proportion of animals after section of the nerve on day 2 suggests a rapid transport of virus in the nervous system. Such rapidity is in line with previous reports of movement of virus by intra-axonal transport rather than by the much slower spread by sequential infection of glial cells (for review, see Hill, 1984).

The phenomenon of zosteriform lesions is not restricted to a particular strain of mouse; indeed, it occurs just as efficiently in outbred or NIH inbred animals and also in DBA mice (D. M. Altmann, personal communication). Nor is it restricted to particularly young animals since those inoculated when 8 weeks old were just as susceptible as 4-week-old mice (a confirmation of the report of Sydiskis & Schultz, 1965). In contrast, mortality was much decreased in 8-week-old mice, which emphasizes that separate controls must exist for these two facets of infection. Even strains of very low virulence (for instance KOS) induced zosteriform lesions efficiently, an observation that prompted the comparison between titres in tissues infected with KOS and those infected with SC16. The lack of discernible differences suggests that the differing virulence must arise from mechanisms so far untested.

Many strains of HSV-2 are extremely neurovirulent in mice (Hill et al., 1975) which often die before zosteriform spread can develop. However, with relatively avirulent strains (e.g. AR11) zosteriform spread of lesions occurred just as frequently as with HSV-1.

Even though the inoculated dose from a drop of suspension with $1 \times 10^5$ p.f.u. of virus placed on the skin must be considerably less than the $3 \times 10^5$ p.f.u. injected in previous work (Hill et al., 1975), latent infection resulted in a higher proportion of animals inoculated with the scarification technique. This might arise because virus replicates more and has better access to nerve endings when introduced into the epidermis by scarification rather than by s.c. injection.

It is not yet known which neurons within the cervical ganglia serve different areas of skin. However, recurrent disease can be induced in the skin of the pinna in mice inoculated in the
Zosteriform spread of HSV

1485

neck. Thus, latent infection must be established in neurons that do not serve the site of inoculation. This confirms our similar result that after corneal inoculation, latent infection is established in areas of the trigeminal ganglion anatomically separate from that which innervates the eye (Tullo et al., 1982a). Two mechanisms by which such areas of nervous tissue could become latently infected deserve some attention. Firstly, spread within the CNS may be involved in the establishment of latency by the 'back door' route (Tullo et al., 1982b) as well as in development of zosteriform infection. Secondly, the zosteriform lesions themselves can presumably act as a source of virus to induce latent infection in further neurons serving the newly infected area of skin.

Since zosteriform lesions occur 5 to 6 days after inoculation they arise in the face of the developing immune response (Nash et al., 1980; Darville & Blyth, 1982) (a slight advantage towards immunity might explain the 10 to 15% of animals that show erythema without surface lesions). In this respect, as well as in their clinical and histological appearance and short-lived nature they somewhat resemble recurrent lesions (Hill et al., 1982). Moreover, as in recurrent lesions (Hill et al., 1983b) the virus that produces the disease is delivered to the skin via axons without extraneous pathology. Study of zosteriform spread has clearly enhanced understanding of the complexities of primary HSV infection. From preliminary studies it is likely that the zosteriform spread model will have practical use in testing treatments against HSV (Hill et al., 1984). The place of zosteriform spread in the human infection will be much more difficult to ascertain but the distribution of lesions on the external genitalia of women infected with HSV-2 suggests the possibility that such spread is involved (Corey, 1984).

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REFERENCES


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