Spread of Virus and Distribution of Latent Infection Following Ocular Herpes Simplex in the Non-immune and Immune Mouse

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SUMMARY

In both non-immune and immune mice infected with herpes simplex virus the incidence of latent infection of the trigeminal ganglion was related to the severity of ocular virus infection. During primary infection, virus was shown to travel via the ophthalmic part of the ganglion to reach the brainstem, from where centrifugal spread resulted in latent infection of neurones in the trigeminal ganglion which did not serve the site of inoculation. Primary infection also resulted in latent infection of the superior cervical ganglion. Shedding of virus occurred rarely in the tears of animals which had recovered from primary disease. In immune mice, spread of virus resulted in a much lower incidence of latent infection and that occurred only in ophthalmic neurones.

INTRODUCTION

The range of signs which result from herpes simplex virus (HSV) infection of the mouse eye is considerable and, with the aid of slit-lamp examination, culture of the tear film and monitoring of the corneal reflex, it can be documented in detail (Tullo et al., 1982a, b). Latent infection of neurones in sensory ganglia follows infection of the periphery (Cook et al., 1974; McLennan & Darby, 1980), and such infection of the trigeminal and superior cervical ganglia is a well-documented consequence of ocular HSV infection in the rabbit (Nesburn et al., 1972; Martin et al., 1977) and in the mouse (Knotts et al., 1974; Walz et al., 1974; Kristensson et al., 1979). In the present study, the spread of virus in the nervous system to these sites has been documented. A correlation has been made between the severity of eye infection and the frequency and the site of latent infection in the ophthalmic, maxillary and mandibular parts of the trigeminal ganglion of non-immune and immune mice.

METHODS

Animals. Male outbred Swiss mice were used throughout. Both eyes of all mice were examined with the slit-lamp microscope before inoculation; animals that showed any ocular abnormality were excluded. Following inoculation, mice were transferred from sawdust to shredded newspaper bedding to avoid the risk of ocular damage from sawdust.

Virus. Stock suspensions of the SC16 strain of HSV type 1 (Hill et al., 1975) were prepared from infected Vero cells.

Inoculation of animals. Mice were anaesthetized by intraperitoneal injection of sodium pentobarbitone. For primary infection, 5 μl of virus suspension in growth medium (Hill et al., 1975) containing 0.5 × 10⁴ to 2.8 × 10⁴ p.f.u. was placed on the left eyes of 8-week-old mice. For each mouse 10 linear corneal scarifications and a further 10, perpendicular to the first, were made with a 26 gauge needle. Secondary infection was studied in mice which had been infected in the right ear when 4 weeks old (Hill et al., 1975) and then 4 weeks later, animals were infected in the left eye as described above with an inoculum containing 0.5 × 10⁶ p.f.u.

Isolation of virus from eye washings. A 20 μl amount of growth medium was irrigated and aspirated 10 times upon the left eyes of anaesthetized mice and placed immediately on to a Vero cell monolayer in plastic dishes.

Isolation of infectious virus from tissues. Animals were killed by intraperitoneal injection of pentobarbitone and exsanguinated by evisceration. The corneas and superior cervical ganglia were removed from the mice and the trigeminal ganglia were divided in situ into three parts: ophthalmic (I), maxillary (II) and mandibular (III) using
separate sterile instruments for each part (see Fig. 1). Each sample was placed in 0.5 ml growth medium and ground in a glass grinder and then frozen and thawed three times before placing 50 μl of the suspension on to monolayers of Vero cells. Plates were incubated for 2 days, then fixed and stained and the virus plaques counted. The amount of virus isolated from different specimens was quantified by the scoring system: 0, no virus; 1, 1 to 49 p.f.u.; 2, 50 to 100 p.f.u.; 3, >100 p.f.u.

Detection of latent infection in nervous tissue. Samples were placed in 0.5 ml growth medium in a bijou bottle and incubated at 35 °C for 5 days. Samples were ground and 50 μl amounts of the suspension were placed on Vero cell monolayers and incubated at 35 °C in 5% CO₂ atmosphere for 2 days before fixing and staining, and the virus plaques counted.

Since only one-tenth of the tissue suspensions was sampled for isolation of virus, the sensitivity was approx. 10 p.f.u./sample.

RESULTS

The clinical signs of primary and secondary infection, and the classification of disease according to site and severity have been described in detail elsewhere (Tullo et al., 1982a).

Attempts to isolate virus from the cornea after recovery from primary infection

Thirty-six mice were killed 23 to 115 days after infection and examined for the presence of infectious virus in the cornea, but none was found.

Isolation of HSV from eye washings

(i) After resolution of primary infection, the left eyes of 59 mice (all of whom had shown lid disease only) were examined for the spontaneous shedding of virus during the period 23 to 113 days after inoculation on a total of 531 occasions. Virus was isolated on a single day from each of four mice on days 38, 60, 80 and 113 after inoculation. Apart from the last mouse, which was washed only once, no virus was isolated from any mouse on the day before or after the occasion when virus was cultured. Only one to five plaques were detected on each of the four occasions. Of 42 mice subsequently examined, 26 (62%) were found to be latently infected in the ophthalmic part (I) of the trigeminal ganglion (TG), and 13/32 (41%) in the superior cervical ganglion (SCG). Three of the four mice which shed virus were examined for latent infection. In one mouse no latent infection was demonstrated. In the second, latent infection was demonstrated in part I of the TG, and in the third mouse it was also present in the SCG. The fourth mouse was examined for the presence of infectious virus in the cornea, TG and SCG at the time of shedding in tears but none was found.

(ii) After resolution of secondary infection, the left eyes of 47 mice (all of which had shown mild or severe keratitis) were washed 56 to 70 days after infection, on between 6 and 10 separate days. Virus was never isolated on a total of 341 occasions. Of 29 mice subsequently examined, five were found to be latently infected in part I of the TG and none in the SCG (see Table 3).

Spread of virus through nervous tissues during acute disease

Groups of two to six animals were killed at daily intervals following infection in order to trace the spread of virus in the nervous system. Only mice with eye disease and without neurological signs of disease were selected.
Table 1. Isolation of HSV from the ipsilateral trigeminal ganglion and brainstem of mice after infection of the cornea*

<table>
<thead>
<tr>
<th>Time after infection (days)</th>
<th>Trigeminal ganglion</th>
<th>Brainstem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ophthalmic (I)</td>
<td>Maxillary (II)</td>
</tr>
<tr>
<td>1</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>2</td>
<td>2/4 (1.0)†</td>
<td>0/4</td>
</tr>
<tr>
<td>3</td>
<td>2/4 (1.0)</td>
<td>0/4</td>
</tr>
<tr>
<td>4</td>
<td>4/4 (2.5)</td>
<td>1/4 (1.0)</td>
</tr>
<tr>
<td>5</td>
<td>3/3 (2.0)</td>
<td>2/3 (1.0)</td>
</tr>
<tr>
<td>6</td>
<td>3/3 (2.7)</td>
<td>3/3 (1.7)</td>
</tr>
<tr>
<td>7</td>
<td>3/3 (1.7)</td>
<td>2/3 (1.5)</td>
</tr>
<tr>
<td>9</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>11</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* Eight-week-old male outbred Swiss mice were used and the left cornea of each mouse was scarified through suspension containing 0.6 × 10^6 p.f.u. All mice showed signs of keratitis and most had lid margin disease. None showed signs of neurological disease.
† Numbers in parentheses are the mean score of specimens from which virus was isolated.

Table 2. Latent infection after primary ocular infection

<table>
<thead>
<tr>
<th>Site of latent infection</th>
<th>Trigeminal ganglion</th>
<th>Superior cervical ganglion</th>
<th>Brainstem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signs of disease</td>
<td>Ophthalmic (I)</td>
<td>Maxillary (II)</td>
<td>Mandibular (III)</td>
</tr>
<tr>
<td>None</td>
<td>4/16 (25)†</td>
<td>0/16</td>
<td>0/16</td>
</tr>
<tr>
<td>Lid disease</td>
<td>7/11 (64)</td>
<td>5/11 (45)</td>
<td>1/11 (9)</td>
</tr>
<tr>
<td>Lid disease + transient mydriasis or corneal signs</td>
<td>1/1 (100)</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Lid disease + irreversible corneal signs</td>
<td>13/15 (87)</td>
<td>6/15 (40)</td>
<td>7/15 (47)</td>
</tr>
</tbody>
</table>

* All samples were ipsilateral to the site of inoculation.
† Results are expressed as virus isolated/total tested with percentage in parentheses.
‡ ND, Not done.

Primary infection

Infectious virus was first isolated from the ophthalmic part (I) of the TG and the ipsilateral brainstem on day 2 after inoculation. On day 4, virus first appeared in the maxillary part II. Not until day 5 was virus isolated from the mandibular part (III), at which time it was also first isolated from the contralateral brainstem (Table 1).

Secondary infection

Attempts were made to isolate virus on days 1 to 4 and day 7 from the TG only. No virus was cultured except on day 2 when virus was isolated only in part I from three of six mice tested.

Latent infection

Thirty-six mice were killed 23 to 115 days after primary infection and examined for the presence of infectious virus in the TG parts I to III and the SCG, but none was found.

After primary infection

Latent infection was sought 23 to 62 days after primary infection. The incidence of latent infection was related to the severity of eye disease (Table 2). Latent infection in the ophthalmic part (I) of the TG (but not elsewhere) occurred even in the absence of signs of infection. After
severe disease latent infection occurred frequently in all three parts of the TG. When signs of ocular infection had been apparent the incidence of latent infection of the SCG was the same irrespective of the severity of disease. When periocular spread of disease occurred in association with severe keratitis the incidence of latent infection in the contralateral TG was 11/17 (65%). On the rare occasions in primary infection when severe keratitis occurred without lid margin involvement, latent infection was detected in 1/4 mice in part I of the contralateral TG.

After secondary infection
In addition to latent infection of the spinal ganglia (Hill et al., 1975), initial infection of the right ear led to latent infection of the mandibular part of the right TG in 3/22 (14%) mice, but not of the left (contralateral) TG.
When subsequent infection of the left eye produced severe keratitis, latent infection was detected 47 to 60 days after infection in part I of the left TG in 7/24 (29%) mice and in the part III in 1/24 (4%) mice. Latent infection did not occur in the SCG and was rare in the TG after mild keratitis (Table 3).

DISCUSSION
Results of the experiments reported here confirm that, in the mouse, latent infection of the TG and SCG follows ocular infection with HSV. Our previous study indicated that the severity of eye disease could be correlated with the frequency with which virus could be isolated from eye washings during the acute infection (Tullo et al., 1982a). This study shows that a correlation also exists between severity of peripheral infection and the likelihood of consequent latent infection. Although shedding of virus in the tears of humans (Kaufman et al., 1967) and rabbits (Nesburn et al., 1972; Kwon et al., 1981) has been shown to occur in the absence of clinical disease, this phenomenon has not previously been demonstrated in the mouse (Knotts et al., 1974; Hill et al., 1981). In this study, small amounts of virus were occasionally isolated from mice that had recovered from lid disease.

In previous reports on latency following ocular herpes, the TG has been examined as a whole (Knotts et al., 1974; Kristensson et al., 1979). Since ophthalmic neurones represent only part of this large sensory ganglion (Arvidson, 1979), and each part supplies a different area (Gregg & Dixon, 1973), it was thought desirable to separate the ganglion into its three component parts. Although division of the ganglion into these parts cannot be absolute, it was felt that part I contained all ophthalmic neurones plus some maxillary neurones, part II contained only maxillary neurones, and part III contained only mandibular neurones. In particular, it is certain that just as ophthalmic and mandibular regions of the face are not adjacent, neither are the trigeminal neurones supplying them (Mazza & Dixon, 1972).

The highest incidence of latent infection in the TG occurred in mice with the most severe disease; virus was also isolated more frequently from such animals. Thus, the increased incidence of latent infection might result from the greater availability of virus to enter the central nervous system (CNS). As the severity of disease increased so did the likelihood of latency in non-ophthalmic trigeminal neurones. The finding of latent infection, particularly in the mandibular part of the ganglion, was unexpected and might be explained in a number of ways.

Although haematogenous spread of virus has been demonstrated (Cook & Stevens, 1973), the
amount of virus entering the blood as a result of ocular infection is minimal (Knotts et al., 1974) and is unlikely to have been responsible for virus reaching mandibular neurones.

In some mice disease spread to the skin of the forehead and cheek. In these instances, latent infection in the maxillary part could have been consequent on spread of virus to the ganglion in nerves supplying the lower eyelid and cheek. However, considerable care was taken to look for lesions in this region of the lower lip and jaw, i.e. those areas supplied by the mandibular nerve. On the rare occasions when such lesions were seen, the mice were excluded from the study. Spread of virus in tears via the nasolacrimal duct almost certainly brings virus into contact with the mucous membrane of the nasopharynx which is supplied by maxillary and mandibular nerves. However, the dilution of virus in tears and nasal secretions and the requirement (at least in the cornea) for damage to epithelium in order to produce disease (Tullo et al., 1982a, b) make infection at this site unlikely.

Spread of virus from the ophthalmic part of the ganglion to other neurones is unlikely as there are no intraganglionic connections in sensory ganglia. Moreover, in adult mice both satellite cells which cover the neuronal cell body and Schwann cells which surround the axon, are relatively resistant to infection by HSV (Cook & Stevens, 1973; Hill & Field, 1973), and are likely to constitute a barrier to such spread.

Latent infection could have been established in non-ophthalmic parts of the ganglion by centripetal spread of virus to the brainstem (via ophthalmic neurones) and thence by centrifugal spread to maxillary and mandibular parts. The sequence in which virus was isolated from different sites in the nervous system during primary infection suggests that this was the most likely course of events. The occurrence of latent infection in the contralateral TG is further evidence for outflow of virus from the CNS. Latent infection of the contralateral TG has also been demonstrated after corneal infection in the guinea-pig (Tenser & Hsiung, 1977) and in contralateral spinal ganglia of mice infected in the flank (Knotts et al., 1973). Two factors which would aid the lateral spread of HSV within the CNS are that glial cells within the CNS are susceptible to infection with HSV (Townsend, 1981), and that a single oligodendrocyte is related to several axons (Bunge, 1968). The extensive demyelination which has been demonstrated in the CNS and not the peripheral nervous system following ocular infection (Townsend, 1981), emphasizes the different response to HSV in these parts of the nervous system. In spite of the presence of infectious virus in the CNS during the acute infection, latent infection was not demonstrated in the brainstem, even in mice which had suffered severe keratitis. Other workers have demonstrated latent infection in the CNS of mice and rabbits (Knotts et al., 1973) but at a much lower incidence than in sensory ganglia. This may reflect a difficulty in showing latency in the CNS rather than a real difference in incidence since virus DNA has been demonstrated in the brains of 6/20 mice (Cabrera et al., 1980) and 7/11 humans (Fraser et al., 1981).

Earlier studies showed that in mice previously infected in the skin virus was cleared rapidly from the tear film after ocular infection despite a 100-fold increase in the inoculum compared to that used to initiate primary infection in the eye (Tullo et al., 1982a). Unlike primary disease, ocular herpes in mice immune after infection of the ear (Nash et al., 1980; Darville & Blyth, 1982) consisted of a reversible keratitis. The reduction in the severity of eye disease was reflected by a reduction in the frequency and distribution of latent infection. Thus, latent infection occurred almost exclusively in the ophthalmic part of the TG and in less than one-third of mice which had shown the most marked keratitis; it was rare after mild infection. This again indicates a correlation between severity of peripheral disease and subsequent latent infection, a correlation that might be expected since, in immune animals, virus is quickly eliminated from the eye so that entry into nerve endings is less likely (Tenser & Hsiung, 1977). The restriction of infectious virus to part I of the TG (where it was found only on day 2) is consistent with the absence of neurological signs, death and the limited distribution of latent infection. It confirms the findings of others concerning the restrictive effect of previous exposure to HSV on distribution and frequency of latent infection (Price et al., 1975; Klein et al., 1978; Walz et al., 1976).

The results reported here show how virus may establish latent infection in neurones that do not supply the site of primary infection. We also have evidence that in mice, following primary inoculation of the lower lip, virus not only reaches mandibular neurones but establishes latent
infection in ophthalmic neurones (Tullo et al., 1982b). The results of Lonsdale et al. (1979) and Gerdes et al. (1981) provide evidence of such spread in man. By restriction enzyme analysis they showed that in a single individual isolates from different sites were of the same strain of HSV. Thus, the single exposure to oropharyngeal infection in childhood (Buddingh et al., 1953) may be the origin of virus causing subsequent eye disease [which has a much lower incidence (Norn, 1970) and occurs later in life (Wilhelmus et al., 1981)].

The difficulty of establishing latency in the immune mouse argues against the likelihood of exogenous infection or auto-inoculation as a source of virus causing keratitis in the immune individual. On the other hand, the results indicate that subclinical primary infection of the eye can also result in a low incidence of latent infection in ophthalmic neurones.

Our previous studies on ocular infection with HSV in non-immune and immune animals indicated that the range of signs could be documented in detail. The present study has shown that the severity of these signs gives a reliable indication of the site and frequency of associated latent infection. It is expected that this will prove valuable in future experiments designed to study the mechanisms underlying recurrent eye disease.

REFERENCES


Latent infection after ocular herpes in mice


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