Neuronal Propagation of HSV1 from the Oral Mucosa to the Eye

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PURPOSE. To identify possible neuronal pathways leading to herpetic ocular disease after primary oral infection in mice.

METHODS. The SC16 strain of herpes simplex virus (HSV)-1 (10⁶ plaque-forming units) was injected into the mucocutaneous border of the left upper lip. Animals were killed 2 to 10 days postinoculation (DPI). Spread of the virus in neural structures was studied by immunohistochemistry.

RESULTS. HSV1 first replicated at the site of inoculation and then at the superior cervical ganglion (at 2 DPI). The trigeminal ganglion and the facial nerve fibers were infected by 4 DPI. Infection of the ciliary body and iris occurred at 6 DPI, together with several brain stem nuclei belonging to the autonomic or sensory pathways. Between 8 and 10 DPI, the neural infection gradually cleared up, except for the ipsilateral sympathetic ganglion, and ipsilateral keratitis appeared in some animals.

CONCLUSIONS. The pattern of viral dissemination in this mouse model suggests that infection of iris and ciliary body results from transfer of virus in the superior cervical ganglion from sympathetic neurons innervating the lip to neighboring neurons innervating the anterior uvea. Later, zosteriform spread of virus from the trigeminal system may have contributed to the clinical and histologic findings. (Invest Ophthalmol Vis Sci. 2000;41:2600–2606)

Herpes simplex virus (HSV)-1 induces recurrent oral and ocular disease. The primary infection generally occurs during childhood after contact with infected lesions or saliva. Symptomatic primary infections mostly involve the oropharyngeal tract and rarely the eye (less than 1% of cases).1 In contrast, ocular symptoms related to viral reactivation are frequent, affecting 21 of 100,000 persons per year in Western countries.1,2 These epidemiologic data suggest that oropharyngeal primary infection may lead to eye disease much later in life,3 a hypothesis supported by some experimental data. For instance, HSV1 inoculation in the lower lip of mice led to latent infection of the trigeminal ganglion (TG), including its ophthalmic part.4 Other experiments have confirmed that HSV1 can establish latent infection in neurons that do not supply the site of primary infection.2,4 After inoculation in the animal’s snout (ophthalmic branch area), the virus was found in the TG and superior cervical ganglion (SCG), then in the cornea, iris, and ciliary body.5–9 Conversely, inoculation in cornea or anterior eye chamber led to latent infection in the whole TG and SCG.4,10

Human herpetic iritis can occur with no signs of simultaneous or past keratitis—that is, without involvement of the trigeminal system. Such isolated herpetic anterior uveitis appears to be caused by viral spread through neuronal structures that supply the iris and the ciliary body—namely, the autonomic pathways. Postmortem studies have shown evidence of herpetic latency in the central nervous and autonomic systems in humans.12–15 In this article, we present a murine model of anterior uveitis without concurrent keratitis resulting from oral HSV1 inoculation.

MATERIALS AND METHODS

The wild-type SC16 strain of HSV116 was cultured in infant hamster kidney cells, concentrated, and kept at −80°C until use. Concentrates were thawed, diluted, and titrated in Vero cells (African green monkey kidney cells) before inoculation.

Six-week-old inbred BALB/c female mice were used for all experiments. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. Equithesine anesthesia17 was used.

Under microscopic observation, 1 μl of HSV1 suspension was slowly injected into the subepithelial layers at the mucocutaneous border of the left upper lip, using a glass micrопette connected to a pressure delivery device.18 Each animal was examined daily to detect ocular infection (blepharitis, conjunctivitis, keratitis, or iritis).

Twenty-two mice were inoculated with 10⁶ plaque-forming units (PFU) of the SC16 strain of HSV1. Three of them were randomly chosen and killed 2 and 4 days postinoculation (DPI). Four mice died between 6 and 7 DPI. Mice with clinical ocular...
disease were killed by groups of three at 6, 8, and 10 DPI. Three mice with no clinical ocular disease were killed at 9 DPI. Under terminal anesthesia, mice were transcardially perfused with phosphate-buffered saline (PBS), 4% paraformaldehyde in PBS, and 20% sucrose in PBS. The skull was decalcified for 7 days at 4°C in 0.1 M EDTA dissolved in PBS, stored in PBS containing 20% sucrose for 24 hours at 4°C, and finally frozen. The spinal cord and SCG were frozen 24 hours after storage in PBS-20% sucrose. Frontal cryosections (30 μm) of the entire brain were collected into three parallel series, each one containing every third section. Two series were stained by the peroxidase-antiperoxidase method using a metal-enhanced diaminobenzidine kit as substrate (Pierce, Rockford, IL). The third series was kept in reserve. The sections were counterstained with Giemsa blue, washed, dried, and mounted.

HSV1 infection was quantified during two independent microscopic evaluations (performed by ML). For each animal, two of three series were examined (approximately 500 of the 750 total sections per mouse). Each slide was analyzed independently at random to limit the subjective nature of the evaluation. Levels of high interest were also examined by PK, GU, and AF. Only one series of sections from sham-infected animals was examined (approximately 250 sections). When the infection progressed, labeled cells formed foci and could no longer be counted. Therefore, we used a semiquantitative scale: mild, moderate, and severe infection (see legend of Table 1).

**RESULTS**

Sham-infected mice showed no signs of HSV1 infection. Among the 22 inoculated mice, 4 died between 6 and 7 days, and 18 were killed from 2 to 10 DPI. Immunohistochemical staining of several key structures is illustrated in Figures 1 and 2. The spatial and temporal pattern of viral spread is summarized in Table 1 and Figure 3.

### Infection of the Labial Mucosa

At 2 DPI, all the mice had a cold-sore–like lesion at the site of inoculation (Fig. 1A). HSV1 immunostaining was observed in the intra- and subepithelial layers of the mucosa in all animals killed from 2 to 6 DPI, but not later. Other portions of the lips, mouth, and tongue were not labeled.

### Infection of the Eyes

Ocular disease was present from 6 DPI in the four mice that died of the infection and in 13 of the 16 mice (81%) that survived for at least 6 DPI. Immunolabeling of the iris and ciliary body of the left eye appeared at 6 DPI (Fig. 1G) but decreased from 8 DPI onward. The principal duct of the left lacrimal gland was labeled in only one mouse (6 DPI). Stromal and epithelial keratitis was noted in three animals (one at 8 DPI and two at 10 DPI).

The conjunctival epithelium was not labeled. The retinas of the left eyes and the whole right eyes remained free of labeling in all animals (Fig. 1H). No labeling was found in the eyes of the three mice with no clinical signs of ocular infection when killed at 9 DPI.

### Infection of Autonomic Pathways

The first infected individual neurons were detected in the left SCG (sympathetic relay) in three mice killed at 2 DPI. The number of labeled cells then increased (more than 100 infected cells per ganglion at 4 DPI; Fig. 1B) and remained high until 10 DPI. The left sympathetic intermediolateral cell group in the spinal cord (cervical and first thoracic segments), including neurons and glial cells, was maximally labeled at 6 DPI (Fig. 1D) and remained labeled until 10 DPI in two mice. The right intermediolateral cell group was also infected in two of three dishes.
mice at 6 DPI, but not later. The right SCG was infected in one of three mice at 6 and 8 DPI.

The left pterygopalatine (PPG) and ciliary ganglion (CG; Fig. 1E; parasympathetic relays) were infected at 6 DPI. At this time, Edinger–Westphal nuclei were also bilaterally labeled (Fig. 2C). Only two of the three mice showed infection of the CG at 8 DPI, but none at 10 DPI.

In one of the three mice with no clinical ocular disease, some labeled cells were found in the left SCG and intermediolateral cell group, but parasympathetic pathways were not labeled.

Infection of Sensory and Motor Pathways

Immunolabeled cells were detected in the maxillary part of the left TG from 4 DPI (Fig. 1C). At 6 DPI, the infected neurons increased in number and were present in all three parts of the left TG and the left spinal trigeminal nucleus (Figs. 2A, 2D, 2E).

Acute infection of the sensory pathways reduced afterward. At 10 DPI, only a few labeled cells were seen in the left TG. Rare infected motoneurons were seen in the left facial motor nucleus at 4 and 6 DPI, but not later.

In mice with no clinical ocular disease, trigeminal neurons were not labeled. Weak staining of glial cells in the left sensory root of the trigeminal nerve and in the fibers of the left facial nerve was seen in three and two mice, respectively, suggesting that some infection previously occurred in related sensory and motor neurons.

Infection of Other Brain Nuclei

No immunolabeling was seen in other brain nuclei until 6 DPI. At this time, in all animals, scattered infected cells were found bilaterally in the nucleus of the solitary tract, area postrema, locus ceruleus, paraventricular nucleus, and zona incerta and unilaterally in the left amygdaloid complex (Figs. 2A through
2E). One mouse also had labeled cells in the left suprachiasmatic nucleus. The other two mice were also infected in the right ventral posterior part of the thalamus (Fig. 2B) and the right parabrachial nucleus. One of them was also labeled in the superior colliculus (Fig. 2C) and the other one in the mediodorsal and lateral groups of cells within both suprachiasmatic nuclei (Fig. 2A).

At 8 DPI, the pattern of infection in the brain was similar, although labeled cells were less numerous. At 10 DPI, only one mouse showed rare labeled cells in the zona incerta and the ventromedial and posterior parts of the thalamus.

**DISCUSSION**

We traced the propagation of HSV1 strain SC16 on serial histologic sections after inoculation into the mucocutaneous border of the left upper lip of mice. The use of inbred BALB/c
mice, known for their susceptibility to herpes infection, and of the highly neurovirulent SC16 strain of HSV1\(^{16}\) (10\(^6\) PFU) allowed us to observe ocular disease in 81\% of inoculated mice. Smaller inocula (10\(^{-2}\)–10\(^{-4}\) PFU) induced lower rates of eye infection (data not shown).

In all animals with ocular disease after primary oral mucosal infection, the ipsilateral iris was HSV1-positive from 6 DPI. At 8 and 10 DPI, the ipsilateral cornea was also infected in three of six mice. These results show the reproducibility of HSV1 propagation from the oral mucosa to the anterior uvea in our animal model.

We chose to inoculate small volumes of virus within the superficial layers of the lip rather than using a scarification method to minimize the risk of autodissemination by scratching. Although the latter could not be excluded, because a cold sore lesion developed in all mice at 2 DPI, it could not be the origin of ocular infection, because herpes conjunctivitis was absent, and keratitis occurred only sporadically. The observation that the iris was always infected before the cornea suggests that eye disease resulted from propagation of the virus within the nervous system.

The main pathways of viral propagation are schematically summarized in Figure 3. From the upper lip, the virus propagated first to the ipsilateral SCG (2 DPI) and replicated in sympathetic neurons innervating the lip. After replication, mature virions budding out from these neurons infected neighboring cells by local (nonsynaptic) spread, giving rise to multiple foci of infection at 4 DPI. At 6 DPI, the entire SCG was infected. Neurons infected by local spread in the SCG thus included those innervating the ipsilateral iris and ciliary body, and most likely represented the anatomic support for propagation of the virus to the anterior uvea. Although the propagation of HSV1 in the nervous system is mainly subserved by transneuronal transfer between connected neurons, HSV1 can also infect nonneuronal cells.\(^{19,20}\) Nonneuronal infection can be either abortive\(^{21–23}\) or productive\(^{24}\) leading to the infection of neighboring neurons.\(^{20}\)

This is correlated to the neuroinvasiveness of the HSV1 strain and the susceptibility of the animal host.\(^{20}\) For example, the McIntyre-B strain does not productively infect satellite cells within the TG of BALB/c mice, whereas mature virions are found in these cells after infection with the F strain.\(^{18,19,43}\) The occurrence of local transfer was highlighted by the staining of glial cells—for instance, in the white matter of the superior colliculus, zona incerta, and ventral posterior thalamus.\(^{40,41}\) The labeling in deep layers of the superior colliculus, zona incerta, and ventral posterior thalamus is in keeping with the connections of these structures with the sympathetic intermediolateral cell group in the spinal cord,\(^{33–35}\) which may have been infected as early as 5 DPI (as suggested by heavy labeling at 6 DPI). The infection of the locus ceruleus, suprachiasmatic nuclei, and amygdaloid complex could be explained by their connections with the nucleus of the solitary tract and/or the paraventricular nucleus.\(^{34,36–39}\) The labeling in deep layers of the superior colliculus, zona incerta, and ventral posterior thalamus is in keeping with the connections of these structures with the sympathetic intermediolateral cell group in the spinal cord,\(^{33–35}\) which may have been infected as early as 5 DPI (as suggested by heavy labeling at 6 DPI). The infection of the locus ceruleus, suprachiasmatic nuclei, and amygdaloid complex could be explained by their connections with the nucleus of the solitary tract and/or the paraventricular nucleus.\(^{34,36–39}\)

This delay makes it unlikely that the virus traveled to the ganglion by retrograde transport from peripheral parasympathetic nerve endings. One possibility is that parasympathetic neurons were infected by local transfer from sympathetic and/or trigeminal fibers traveling through the PPG (Fig. 3)\(^{31,32}\) after replication in SCG and TG neurons. Similarly, the parasympathetic CG neurons could be infected by local transfer from the sympathetic fibers and/or sensory (nasociliary) fibers that cross the ganglion. Alternatively, the virus may have already arrived in the iris by 5 DPI (a time that was not examined) and then propagated retrogradely to the CG.

At the time of maximal infection (6 DPI), the nucleus of the solitary tract, area postrema and paraventricular hypothalamic nuclei were all labeled. Such a pattern of spread is in agreement with the connections of these structures with the sympathetic intermediolateral cell group in the spinal cord,\(^{33–35}\) which may have been infected as early as 5 DPI (as suggested by heavy labeling at 6 DPI). The infection of the locus ceruleus, suprachiasmatic nuclei, and amygdaloid complex could be explained by their connections with the nucleus of the solitary tract and/or the paraventricular nucleus.\(^{34,36–39}\) The labeling in deep layers of the superior colliculus, zona incerta, and ventral posterior thalamus is in keeping with the connections of these structures with the sympathetic intermediolateral cell group in the spinal cord,\(^{33–35}\) which may have been infected as early as 5 DPI (as suggested by heavy labeling at 6 DPI). The infection of the locus ceruleus, suprachiasmatic nuclei, and amygdaloid complex could be explained by their connections with the nucleus of the solitary tract and/or the paraventricular nucleus.\(^{34,36–39}\)

From 6 DPI onward, we observed a decrease in the number of HSV1-immunolabeled cells in most infected structures, except in sympathetic pre- and postganglionic neurons. This may be related to the clearance of heavily infected cells by the immune system. Indeed, such a response has been shown to occur as early as 3 DPI.\(^{44,45}\) Alternatively, HSV1 become latent in sensory and autonomic neurons connected to the eye.\(^{3,5,6,10,46}\) The multiple sites of HSV1 infection observed in some classes of sensory fibers.\(^{18}\) At 4 DPI, only the maxillary part of the ganglion was labeled, but at 6 DPI the ophthalmic part was also infected. Again, this probably reflected local transfer of virus between the maxillary and ophthalmic fibers. This is in agreement with tracing experiments using another herpes virus\(^{30}\) and studies on HSV1 latency after inoculation in the lower lip.\(^{3}\) The cornea became labeled 2 days after the iris, probably as a result of viral spread through the anterior chamber or through the sensory axons (ophthalmic fibers) rather than through rare sympathetic endings located in the cornea.\(^{31,32}\)

Parasympathetic neurons in the PPG were not labeled until 6 DPI (i.e., 4 days later than the sympathetic neurons in the SCG), even though some parasympathetic efferents of the PPG supply the salivary glands of the oral mucosa.\(^{31,32}\) This delay makes it unlikely that the virus traveled to the ganglion by retrograde transport from peripheral parasympathetic nerve endings. One possibility is that parasympathetic neurons were infected by local transfer from sympathetic and/or trigeminal fibers traveling through the PPG (Fig. 3)\(^{31,32}\) after replication in SCG and TG neurons. Similarly, the parasympathetic CG neurons could be infected by local transfer from the sympathetic fibers and/or sensory (nasociliary) fibers that cross the ganglion. Alternatively, the virus may have already arrived in the iris by 5 DPI (a time that was not examined) and then propagated retrogradely to the CG.
our model represent potential sites of HSV1 latency, and offer an explanation for why anterior uveitis may occur without a history of keratitis. Similarly, the staining of paraventricular and suprachiasmatic nuclei, both connected to the retina,31 could explain how retinitis may occur. The role of the suprachiasmatic nuclei in the pathogenesis of retinitis has been suggested in other models.12

In conclusion, the results obtained in this reproducible animal model, which avoids infection of the eye at the time of inoculation, improve our understanding of herpetic uveal infections. Combined with data from previous studies, they suggest that isolated ipsilateral anterior uveitis after intralabial viral inoculation is mediated by local virus transfer in the SCG, from sympathetic neurons innervating the lip to neighboring neurons innervating the anterior uvea.

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References


