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Ocular HSV-1: Is the Cornea a Reservoir for Viral Latency or a *Fast Pit Stop*?

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Abstract

Purpose—To present a review supporting and refuting evidence from mouse, rabbit, non-human primate, and human studies of herpes simplex virus type 1 (HSV-1) concerning corneal latency.

Methods—More than 50 research papers on HSV-1 published in peer-reviewed journals were examined.

Results—Infectious HSV-1 has been found in mouse denervated tissues and in tissues with negative cultures from the corresponding ganglion. However, the different mouse strains have shown varied responses to different strains of HSV, making it difficult to relate such findings to humans. Rabbit studies provide excellent evidence for HSV-1 corneal latency including data on HSV-1 migration from the cornea into the corneoscleral rim and on the distribution of HSV-1 DNA in the cornea. However, the available methods for the detection of infectious HSV-1 may not be sensitive enough to detect low-level infection. Infectious HSV-1 has been successfully isolated from the tears of non-human primates in the absence of detectable corneal lesions. The recurrence of corneal ulcers in non-human primates before the appearance of infectious HSV-1 in tears suggests that the origin of the HSV-1 is the cornea, rather than the TG. Human studies presented evidence of both ganglion and corneal latency.

Conclusion—Understanding HSV-1 disease progression and the possibility of corneal latency could lead to more effective treatments for herpetic keratitis. However, it is unlikely that operational latency in the cornea will be definitively proven unless a new method with higher sensitivity for the detection of infectious virus is developed.

Keywords

Herpes simplex virus type 1; cornea; latency; trigeminal ganglia; latency-associated transcript

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Introduction

Herpes simplex virus type I (HSV-1) DNA has been found in the corneas of patients with chronic herpetic keratitis, [1–5] the tears of asymptomatic patients,[6–10] and in eye bank corneas.[11–13] Three possible explanations for the presence of this DNA in human corneas are operational latency, persistent low-grade infection, and neuronal reactivation of latent HSV. In humans, HSV infection occurs early in life and can be either symptomatic or asymptomatic. Symptomatic infection generally produces ulcerative lesions on a superficial body surface such as the skin or the cornea. Asymptomatic infection involves the retrograde transport of the virus from the nerves at the site of contact to a ganglion in the absence of lesions. The virus remains in the neurons as intact quiescent viral genomes without any obvious pathological effects, a state known as latency (See Table 1 for a list of definitions and references that support the concepts).

HSV can reactivate from a ganglion and travel by anterograde transport to any site in the distribution of that ganglion. This results in the shedding of viral DNA/infectious virus (enveloped HSV virions capable of host cell invasion, replication, cell lysis, and release of infectious progeny, see Table 1), which results in herpetic lesions in some individuals. HSV-1 is generally accepted to become latent in neuronal sites, such as the trigeminal ganglion (TG). However, non-neuronal sites of latency, such as the cornea, have also been proposed.[14–16]

HSV-1 DNA and infectious HSV-1 (used interchangeably with infectious virus) have been found repeatedly in the corneas of latently infected hosts.[5,11–13, 17–29] However, the concept of HSV-1 corneal latency has failed to gain universal acceptance because of the difficulty in proving that the cornea is truly a reservoir for the virus rather than a *fast pit stop* (transient site) along the exit path from a sensory ganglion. There are three main possibilities to explain the presence of HSV-1 in the cornea. These comprise (1) operational latency which in an animal model is characterized by the immediate absence of infectious virus in the cornea or in the TG at the time of sacrifice and the capacity of the excised cornea or TG to produce infectious virus in the cornea after a period of time; (2) persistent low-grade infection which is characterized by viral replication and release of infectious progeny in such small numbers that infectious virus cannot be easily detected by currently available methods, and no clinical lesions are apparent; and (3) neuronal reactivation with downstream shedding which refers to infectious virus/HSV-1 DNA that originates in the neuron but travels to the cornea by anterograde transport.

Human studies have demonstrated that HSV DNA shedding is an ongoing process in the head and neck region, and possibly in the genital region as well.[6, 17, 30, 31] Thus, latency is not absolute because viral replication denoting the production of infectious virus can be missed by the available detection methods due to their very limited sensitivity. Cell culture, a commonly used technique to detect infectious virus, has been shown to have a sensitivity of 2% or less for the detection of HSV-1.[32]

Although it may not be plausible to definitively prove corneal latency, it is important to study HSV-1 corneal shedding and the possibility of the existence of corneal latency. A better knowledge and understanding of the disease state at the time of corneal transplantation could give rise to more effective treatment and help to reduce the incidence of HSV-related graft failure after penetrating keratoplasty (PKP).[14] Also, the cornea is a relatively accessible tissue, and a more detailed evaluation of HSV disease stages and progression can be accomplished through *in vivo* studies if corneal latency is proven.

Sensitivities of Detection Methods

Before discussing the various studies supporting or refuting HSV corneal latency, it is pertinent to review the strengths and limitations of the tests used to detect HSV-1 DNA and infectious HSV-1. The most sensitive test to detect HSV-1 DNA is polymerase chain reaction (PCR) with a sensitivity of 82% and a specificity of 78%.32 PCR is 1000 to 10,000 times more sensitive than the serology assay for HSV-1 antibody.33 Immunohistochemistry (IHC) detects HSV-1 antigen with a sensitivity of 74% and a specificity of 85%.32 PCR detects the presence of specific segments of the HSV-1 genome and does not imply the presence of the entire genome. PCR does not detect infectious virus, and there is not a one-to-one relationship between the HSV-1 DNA copy numbers and infectious progeny in these tests.

Although cell cultures are the "gold standard" for the detection of the presence of infectious virus, they have a very low sensitivity for detecting HSV-1 compared to PCR assays.[24, 32] One of the reasons for the low sensitivity of cell cultures is the fragility of infectious HSV-1. The lipid envelope is easily disrupted, and a broken envelope renders the virus non-infectious and unable to replicate in cell cultures. Electron microscopy can be used to physically observe viral structures, but has unknown sensitivity, is subject to sampling errors, and provides no information on infectivity.

Viral Factors and Their Relevance to Latency

The latency-associated transcript (LAT) is an RNA transcript from the anti-sense strand that overlaps part of an immediate early gene (ICP-0) of the HSV-1 genome and is suggested to perform several functions, including the inhibition of neuronal apoptosis and the enhancement of reactivation.17, 24, 34–37 LAT has been detected in HSV-1 infected cells during both the replicating (lytic) and non-replicating (latent) stages of infection.[24,37,38] Although LAT is not required to establish latency,[34–36,39,40] it is the only abundantly transcribed viral gene during latency and is a known molecular marker for viral latency.[24] Thus, the presence of LAT in a tissue is commonly used as evidence of HSV-1 latency in that tissue. However, the absence of LAT is not an indication that either the tissue or the cell does not have HSV-1 DNA. In situ hybridization studies of LAT and in situ hybridization studies of HSV-1 DNA have shown that not all latent cells have detectable LAT.[17,37,38] Furthermore, studies of deletion viruses that do not transcribe LAT are HSV-1 DNA positive.[17,38]

Mouse Studies

HSV infection has been extensively studied in mice (Table 2) because of the low cost and availability of different strains of mice. The availability of different mouse strains allows researchers to examine disease progression in genetically specified environments. However, the widespread use of different mouse strains also makes it difficult to relate studies of one mouse strain to studies of a different mouse strain and to studies of other animals.

Nonetheless, the concept of non-neuronal latency was first proposed in mouse studies regarding HSV skin infection in 1979.41 Hill et al.[41] isolated infectious HSV-1 (strain SC-16) from 7 out of 88 (8%) ears of latently infected female Swiss white mice (non-inbred) which received no stimulation and from 9 out of 31 (29%) mice whose ears were stimulated with cellophane tape 4 days before sacrifice. However, the authors failed to report on the origin of the detected virus, which could have come from the ear or the ganglion which innervated the ear.

In addition to the recovery of HSV-1 from the skin, the virus has also been isolated from the eyes of latently infected mice. Abghari et al.18 found infectious HSV-1 (strain STU-4) by culture in 20% (2/10) of the eyes and in 18% (3/17) of the corneas of 6- to 8-week-old mice (DBA/2, BALB/c, C57BL/6). This study also reported that infectious HSV-1 was isolated considerably later from corneal cultures than from TG cultures.[18] Possible explanations for the delay in viral growth could be related to mechanical trapping after reactivation, differences in the process of reactivation in two different tissues, or the number of latent foci in each tissue. This growth delay seen in non-neuronal sites favors corneal latency, as virus from a persistent infection or viral shedding from the TG (or other ganglia) would be expected to grow at a faster rate because replication could begin immediately without the need for reactivation or neuronal anterograde transport.22

Abghari et al.[19] showed the presence of LAT in 25% (5/20) and the presence of ICP-0 (HSV-1 strain STU-4) in 3% (1/34) of specimens taken from latently infected 6- to 8-week-old BALB/c mouse corneas by *in situ* hybridization. This study showed the absence of infectious virus by cell-free and homogenized cell cultures at the time of sacrifice, which were all negative. A possible source of error in the study was that each specimen used for culture was obtained from 2 corneas. One might argue that LAT in each positive specimen could reflect the presence of ICP-0 in one of the corneas, but the disparity in positive specimens (25% vs. 3%) makes this possibility unlikely.[19] Keep in mind that the viral genomic regions for ICP0 and LAT overlap and are on opposing strands of the viral DNA. 38،42

Evidence refuting corneal latency comes from studies that fail to demonstrate reactivation and infectious HSV-1 in the cornea. Easty et al.[3] showed that HSV-1 (strain SC-16) did not persist in the anterior segment of NIH strain mice (non-inbred) when inoculated directly into the cornea. However, Bristol/2 mice (non-inbred) inoculated in the snout did have persistent virus isolated from 8 out of the 20 corneas tested (40%).[3] These findings suggest that either the sensory ganglion is required to establish latency in stromal keratocytes or that the virus thought to be reactivated from the cornea was actually reactivated from the ganglion.

The strain of mouse has been shown to affect the rate of reactivation in mice infected with LAT-positive recombinants of HSV-1.[38] Perng et al.[36,38] reported that BALB/c mice showed no significant difference in reactivation when infected with LAT-positive and LAT-negative strains. However, Swiss Webster mice infected with a LAT negative strain showed a significant decrease in reactivation when compared to those infected with a LAT positive strain.[38] The finding that different mouse strains react differently to viral infection, coupled with the wide variation among mouse strains used for HSV studies, complicates the comparison of mouse studies to those of other animals.

Sawtell et al.[43] have shown that after corneal inoculation in mice, HSV-1 strains can exhibit different rates (high versus low) of reactivating in response to hyperthermia. The HSV-1 strain of 17Syn+ had a high reactivation frequency in mouse tears. A lower reactivation frequency of HSV-1 strain KOS/M (see Table 2) was shown. Sawtell[44] assessed individual neurons for HSV DNA copy numbers and reported a wide variation of 10–1000 copies per neurons for the mouse TG. Also, the percent of neurons positive for HSV-1 DNA in the mouse TG latent with 17Syn+ averaged 7.35% with the range of 1–30% (see Table. 2). These studies demonstrate that one mouse neuron can be assessed with the lower limit of detection at approximately 10 copies of HSV-1 DNA.

In summary, the different mouse strains have shown varied responses to different strains of HSV,[38] making it difficult to relate such findings to humans and rabbits.

Rabbit Studies

Ocular HSV-1 infection and latency have been extensively studied in New Zealand white (NZW) rabbits because their eye size is similar to that of humans and slit-lamp examination is relatively easy, allowing quantitative determinations (Table 3). In addition, the widespread use of NZW rabbits allows reliable comparisons among studies.

O'Brien and Taylor[20] showed HSV-1 (strain RE) latency by the operational definition in 11% (10/88) of male rabbit corneas and in 100% (68/68) of male rabbit TG. In this investigation, no cell-free infectious virus was detected in either the cornea or TG at the time of sacrifice, and the detection technique used was Vero cell culture for corneas and TG.

Zheng et al.[16] demonstrated retrograde migration of HSV-1 DNA from latently infected transplanted corneas to the corneal rims and to the TG of 27% (3/11) and 18% (2/11) of naïve, uninfected rabbits, respectively. After transcorneal epinephrine iontophoresis, there was no migration if infected with a LAT negative strain (17ΔPst, a LAT-negative mutant of $17Syn^{+}$), and successful retrograde migration if the cornea was infected with a LAT+ strain (17Pr, a LAT positive rescuant of 17ΔPst). This study confirmed that HSV-1 DNA was present in the corneas of latently infected rabbits and that it was capable of retrograde infection upon adrenergic stimulation. However, latency in the infected transplant donors was established by the absence of corneal lesions by slit-lamp examination and the absence of detectable viral shedding in the tear film by culture. Moreover, it is possible that the infected rabbits did not have true corneal latency at the time of transplantation, but that the methods of detection were simply not sensitive enough to identify an early reactivation or a low-grade infection.

Cook et al.[21] demonstrated HSV-1 (strain 17) latency in sections of corneal epithelium, keratocytes, and endothelium separately *in vitro*. Latency was established by keeping cultures at a supraoptimal temperature of 41.5° C, and the absence of infectious virus was established by negative cultures and by assays of the culture supernatant at 41.5° C. Reactivation was induced by changing the culture temperature to 37° C, and infectious virus was isolated from 75% (18/24), 58% (14/24), and 66% (16/24) of epithelial, keratocyte, and endothelial cell cultures, respectively. In addition, polyacrylamide gel electrophoresis was used to identify proteins characteristic of acute HSV-1 infection. These proteins were present during incubation at 37° C but absent at 41.5° C. The *in vitro* method excludes the possibility of ganglionic reactivation, as cells were inoculated after microdissection into epithelial, stromal, and endothelial components. Still, corneal latency was not proven because the culture methods used to detect infectious virus may not have been sensitive enough to detect virus at 41.5° C, and persistent virus was observed in 20% (5/24) of epithelial cell cultures.

In another study by Cook et al.,[22] infectious HSV-1 (strains HG-52, 17, McKrae, and R40/2) was isolated from 12% (4/33) of rabbit corneas and 100% (33/33) of rabbit TG 56–97 days post-inoculation. The rabbits showed no clinically apparent corneal lesions and did not shed infectious virus in tears for 14 days before sacrifice. There was a significant delay in viral growth in corneas compared to that in TG. This delay favors corneal latency and argues against TG reactivation and persistent infection as viral growth would be expected in 2–3 days in the latter two cases.[22]

Sabbaga et al.[23] used *in situ* hybridization, tear film, whole-cell, and cell-free cultures to demonstrate HSV-1 (strain RE) corneal latency. HSV-1 DNA was detected in 100% (26/26) of latently infected corneas by *in situ* hybridization at 25, 35, 45, and 60 days post inoculation, and TG whole-cell cultures were 75% (12/16) positive for infectious virus after induction. Prior to induction, tear film, whole-cell explants, and cell-free cultures showed no

infectious virus in the cornea, and cell-free cultures did not detect infectious virus in the TG. Although cell cultures have lower sensitivities to detect HSV-1 than molecular methods such as PCR or *in situ* hybridization,[24, 32] the use of 2 different culture methods in both the cornea and the TG, and 3 methods overall is convincing in documenting the absence of infectious virus. Another important finding in this experiment was the corneal distribution of HSV-1 DNA detected by *in situ* hybridization. During the acute infection (up to post-inoculation day 9), HSV-1 was detected primarily in the epithelium, with a small amount found in the stromal keratocytes, whereas HSV-1 was detected in the epithelium, stroma, and endothelium in comparable amounts during latent infection (post inoculation days 25–60).

Evidence refuting corneal latency comes from studies which do not sufficiently demonstrate the absence of active infection, do not provide stable copy numbers of corneal HSV-1 DNA and RNA, or do not show reactivation with isolation of infectious HSV-1. Cook et al.[24] searched for the presence of LAT and thymidine kinase (TK) DNA and RNA fragments (strains 17 Syn^+ or McKrae) by PCR in infected rabbit corneas and TG. The rabbits had tear film cultures before sacrifice to assess for infectious virus; all were negative. In the HSV-1 DNA studies, 100% (4/4) of ganglia and 60% (3/5) of corneas contained HSV-1 TK and ICP-0 genes (LAT anti-sense). In the RNA studies, LAT was detected in 100% (22/22) of ganglia but only in 9% (2/22) of corneas, and TK was not detectable in any of the ganglia (22) or corneas (22) tested. These results suggest corneal latency is equivocal since the corneas (2/22) which contained LAT transcripts were both from the same rabbit, which was sacrificed 41 days after primary infection with the earliest group of latently infected rabbits. Thus, it is possible that the LAT RNA was residual from the acute infection, as LAT is a late transcript in acute disease.

O'Brien et al.[25] measured the accumulation of LAT and TK in the corneas of latent rabbits to assess for the possibility of HSV-1 (strain RE) corneal latency. The absence of infectious virus in the cornea was assessed by Vero cells. Overall, 57% (17/30) of corneas and 100% (30/30) of the TG contained HSV-1 DNA. LAT was not detected in any of the corneas but was detected in 100% (30/30) of the TG, while TK mRNA was detected in 59% (10/17) of corneas and 76% (23/30) of TG. Viral DNA was present in 100% (16/16) of rabbit corneas in the first 4 months of the study but decreased to 50% (2/4), 12.5% (1/8), and to 33% (2/6) from 20–54 weeks. The decreasing frequency of HSV-1 DNA and the absence of LAT in the cornea compared to the high numbers of HSV-1 DNA and LAT in the TG suggest one or both of the following possibilities: 1) corneal latency is less efficient than neuronal latency and/or 2) corneal latency is of a different molecular mechanism or regulatory system than neuronal latency. In addition, the method of confirming the absence of infectious virus during latency was by Vero cells, so the possibility of a persistent low-grade infection or early reactivation may have been missed and cannot be ruled out as an explanation for the presence of HSV-1 DNA and TK in the cornea.

Openshaw et al.[11] established that the cornea can be a reservoir for HSV-1 DNA by transplanting corneas from HSV-1 (strain McKrae) infected rabbits into naïve rabbits. The experiment demonstrated that the recipients' corneas maintained similar levels of HSV-1 DNA by PCR 5 months after transplantation. However, reactivation failed even after induction attempts with cyclophosphamide and dexamethasone. For 5 months, the recipient rabbits were negative for tear film cultures and seroconversion. Therefore, the study demonstrated that the rabbit cornea was a reservoir for HSV-1, but failed to show reactivation (i.e. recovery of infectious virus), thus failing to prove latency.

In summary, rabbit studies provide excellent evidence for HSV-1 corneal latency including data on HSV-1 migration from the cornea into the corneoscleral rim[16] and on the

distribution of HSV-1 DNA in the cornea.[21, 23] However, the available methods for the detection of infectious HSV-1 may not be sensitive enough to detect low-level infection, and studies with promising findings of corneal LAT mRNA or HSV-1 DNA present in stable numbers have failed to rule out active infection or demonstrate reactivation.

Non-Human Primate Studies

Mice and rabbits have dominated experimental research on ocular herpes because of availability and low cost, but non-human primate studies have been performed to establish a connection between the results of non-primate studies and humans (Table 4). The studies do not test the concept of corneal latency but provide evidence of infectious HSV-1 in monkey corneas.

Varnell et al.45 isolated infectious HSV-1 strain Rodanus from the tear films of 14% (2/14) of squirrel monkeys during recurrent herpetic corneal infections. Rootman et al.46 cultured HSV-1 strain McKrae from the tears of 8 out of 10 (80%) and 7 out of 8 (88%) squirrel monkeys after transcorneal iontophoresis, with one primate in each group shedding virus in tears without any prior stimulation. In addition, Rootman at al.46 reported that the average duration of shedding in squirrel monkeys (1.25 days) was 2.75 days shorter than that of rabbits (4.0 days). Also, the corneal epithelial lesions presented about 2 days before the detection of infectious HSV-1 in tears.46 This finding suggests corneal latency if one assumes that the presence of recurrent corneal lesions indicates the presence of infectious HSV-1. Under this assumption, infectious HSV-1 was present and producing lesions in the corneas 2 days before it was present in the lacrimal glands innervated by the TG.

In summary, infectious HSV-1 has been successfully isolated from the tears of non-human primates in the absence of detectable corneal lesions. The recurrence of corneal ulcers before the appearance of infectious HSV-1 in tears suggests that the origin of the HSV-1 is the cornea, rather than the TG.

Human Studies

HSV-1 corneal latency has been studied in humans (Table 5) as it relates to penetrating keratoplasty (PKP) and herpetic keratitis (HK). Several reports[12, 13, 47, 48] have documented cases of newly acquired HK following PKP. An important question to be posed is, “Did HK come from the donor transplanted cornea or from an endogenous reactivation of HSV-1 in the recipient after induction through PKP?” Remejier et al.12 and Thuret et al.13 reported cases of HSV-1 infection acquired from the donor transplanted cornea after PKP. Both studies documented corneal graft transmission by matching the donor and recipient HSV-1 genomes by PCR, and both donor corneas were clinically normal before transplantation, having normal endothelial assessments at the end of organ culture. Biswas et al.[26] reported 2 cases of HSV-1 infection following PKP, in which neither recipient cornea contained HSV-1 DNA by PCR, but both failed transplants were positive for infectious HSV-1 and HSV-1 DNA by culture, PCR, IHC, and transmission electron microscopy (TEM). Both failed transplants came from donors without any history of herpetic disease and were maintained over 18 days in organ culture prior to transplantation with final endothelial assessments of approximately 2900 cells/mm^2. Each patient underwent successful repeat PKP without recurrence of a clinical HSV infection. Rezende et al.[47] and Remeijer et al.[49] documented cases of newly acquired HK ranging from 6–18 patients following PKP and added that the incidence of ocular herpes is estimated to be 6 to 14.2 times higher in PKP patients compared with the general population.

The detection of HSV-1 in the cornea during HK is important not only to correctly diagnose and treat the condition, but also to study the relationship between HK and corneal latency in

vivo.[1] The mechanism of HK is thought to be a chronic immune response to either a low-level persistent infection or a series of frequently reactivating subclinical infections.[35] Most of the evidence argues against a chronic low-level infection, so the question is whether or not the reactivating infections originate in the ganglia or in the cornea.

Tullo et al.1 and Shimeld et al.2 isolated infectious HSV-1 from 6 of 9 and 2 of 3 (66%) patients undergoing PKP for HK, respectively. Coupes et al.4 cultured infectious HSV-1 from 2 out of 8 (25%) HK patients who had no signs of active disease at the time of surgery. The appearance of virus was thought to be transient because the virus was present in one of the samples only on the day of successful isolation.4 Easty et al.3 isolated infectious HSV-1 from 29.4% (10/34) of patients with a clinical history of HK. Two of the corneas that had infectious virus by culture were examined under TEM prior to culture, and no infectious virions were observed. This finding suggests the absence of infectious HSV-1, but is not definitive as TEM and has a low sensitivity to detect infectious virions. Infectious HSV-1 was only isolated from the central cornea, which favors corneal latency over ganglionic reactivation. Nicholls et al.50 and Beyer et al.[51, 52] suggested that the severing of nerve endings during PKP caused reactivation and release of infectious HSV from the TG, with the greatest numbers of virus being found at the graft-host junction. Cantin et al.5 detected the HSV-1 TK DNA sequence by PCR in over 72% (8/11) of patients with prior HK and in 36% (4/11) of patients without a history of HK. The experiment used PCR to increase the detection sensitivity of HSV-1 DNA from 100 copies to one copy.[5] The authors argue that the high frequency of detection of HSV-1 is unlikely to be of ganglionic origin as asymptomatic viral shedding in humans and animals has been shown to occur in about 10%-30% (not >70%) of corneas with a history of herpetic disease.[5, 24]

The transmission of HSV-1 through PKP is one way to suggest corneal latency, but the presence of HSV-1 DNA and mRNA transcripts in asymptomatic corneas must also be evaluated. Openshaw et al.11 detected HSV-1 DNA sequences for both TK and glycoprotein D (gD) in 9 out of 24 (38%) clinically normal eye bank corneas, while Robert et al.27 showed HSV-1 DNA by PCR in 21% (8/38) of asymptomatic patients who underwent PKP for non-herpetic disease. Van Gelderen et al.28 detected HSV-1 DNA sequences for TK in the recipient corneas of 13 of 78 (17%) patients who underwent PKP for reasons unrelated to herpetic keratitis, including 8 failed corneal transplants without apparent herpetic disease prior to surgery. Kaye et al.29 demonstrated the presence of 3 different HSV-1 DNA sequences in the recipient corneas of 5 out of 10 (50%) non-herpetic PKP patients, of which 3 (30%) also contained LAT as the only mRNA viral transcript present. These studies demonstrate that HSV-1 DNA and RNA are frequently present in asymptomatic corneas. In the last study, the detection of 3 widely spaced HSV-1 DNA sequences in the viral genome implies the presence of the entire DNA genome.29 The discovery of LAT as the only abundant mRNA transcript makes it unlikely that a persistent infection or a reactivation of the TG is responsible for these findings.

In the only human study involving denervation, Hoyt and Billson[42] reported recurrent fever blisters after complete transection of the infraorbital nerve after blow-out fracture. This approach eliminated the possibility of TG reactivation and demonstrated that HSV can survive and reactivate without the support of the TG.

The above studies present a strong case for corneal latency, but there is still a possibility that the PKP recipients either had undetectable HK and/or HSV-1 DNA in their corneas due to reactivation of endogenous HSV-1, or a transient viral presence.

In a study of 450 recipient corneal buttons, 273 donor corneoscleral rims, and 84 clear eye bank corneas, Remeijer et al.[48] showed that increased corneal HSV-1 viral loads correlated

with an increased risk of graft failure in HK patients after PKP but did not negatively influence graft survival in non-HK patients undergoing PKP. In an editorial commentary on this study, Hill and Clement[14] pointed out that the HSV-1 DNA copy numbers found in HK patients were approximately 100 times greater than those found in non-HK patients. However, this observation suggests only that corneal graft failures contained higher numbers of HSV-1 DNA copies. It does not reveal any information about the origin of the HSV-1 DNA found in the cornea.

Remeijer et al.[48, 49] argue that surgical trauma, suture removal, steroids, and immune reactions associated with PKP could trigger endogenous reactivation of HSV-1, resulting in viral DNA or possibly infectious HSV-1 in the cornea. The innervations of the cornea are 300–600 times higher than those of the skin, so disruption of these nerves during PKP can provide a strong stimulus for reactivation.47 Also, many of the patients who developed newly acquired HK after PKP were seropositive for HSV-1 and received corneal transplants that were negative for HSV-1 by PCR.48 In a study with 10 non-herpetic corneas, Kaye et al.29 noted that several viral genes and LAT were present, but none of the "latent" corneas underwent successful reactivation i.e. no detectable infectious virus. Additionally, the study by Openshaw et al.[11] suggested that viral detection was greater in the periphery of the cornea than in the central cornea with 33% (8/24) and 8% (2/24) found in each area, respectively. This finding supports ganglionic shedding more so than corneal latency because the innervations of the cornea occur from the periphery to the central cornea, so it is intuitive that ganglionic shedding would be found more often in the periphery than in the central cornea.

Cell types in cornea as a site of latency

Most studies involving the molecular biology of HSV latency used "whole cornea." However, Cook et al.[21] showed that all three cells types in latent rabbit corneas, that is, the corneal epithelium, keratocytes, and endothelial cells had the characteristics of HSV-1 latency. There have been very limited studies on human endothelial cells and these appear to be an unlikely although possible source of HSV-1 latency. More importantly, since the corneal epithelial cells are highly innervated, the nerve termini are a likely source of HSV-1 DNA. Numerous studies have shown virus particles and HSV-1 DNA in stromal keratocytes. Stromal cells are also highly innervated with nerve terminals that originate from the TG. Thus, both cell types could harbor latent HSV-1 DNA. Conversely, one could argue that all of the measurements of the positive characteristic of HSV-1 in cornea are not due to "true" viral latency but are endpoints of the transport of the virus from ganglionic nerve terminals to the ocular surface.

HSV-1 corneal latency versus HSV-1 neuronal latency (Summary)

All researchers agree that ganglionic neurons are a site of HSV-1 latency. Many studies on TG from rabbits, mice, non-human primates, and humans have provided evidence for this fact. However, there is a controversy concerning the cornea as a site of latency. In science and medicine, it is difficult to prove a negative. Our hypothesis is that HSV-1 is latent in human corneas. One reason is that infectious virus was recovered in some studies from corneal cultures. Additionally, transplanted corneas have been shown to transmit HSV-1.[12–14] Molecular biological studies of the cornea have shown the presence of HSV-1 DNA and HSV-1 transcripts, specifically the LAT transcript. Thus, at least in a few cases, the characteristics used to prove that the ganglionic neurons harbor latent HSV-1 can also be found in the cornea.

Conclusions

HSV-1 corneal latency has long been an important topic. Our hypothesis is that HSV-1 is latent in human corneas. Other possibilities such as persistent low-grade infection and trigeminal reactivation with downstream shedding to the cornea cannot be absolutely ruled out. It is unlikely that operational latency in the cornea will ever be definitively proven unless a new method with higher sensitivity for the detection of infectious virus in the cornea is developed. Nevertheless, it is important to study the HSV-1 disease progression and the real possibility of the existence of corneal latency to improve our understanding of these processes. This knowledge should lead to more effective treatments for herpetic keratitis and decreased graft failures after penetrating keratoplasty.

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Table 1

Terms and Definitions

Term	Definition
Infectious HSV-1 (used interchangeably with infectious virus)	Enveloped HSV-1 virions with a complete genome in unit length configuration capable of host cell invasion, replication, host cell lysis, and release of infectious progeny.[33,34]
HSV-1 DNA (used interchangeably with viral DNA)	The genetic material of HSV-1
LAT (Latency-Associated Transcripts)	The only region of the HSV-1 genome abundantly transcribed during latency. Not required for latency but is a marker for latency.[19,24,41,42]
Latency (general)	Ability of a virus to occupy a host cell without replicating or assembling virions. No infectious HSV-1 can be present.[15,34]
Latency (operational)	Two conditions: **1** Infectious virus cannot be present in the ganglia at time of sacrifice **2** Infectious virus can be successfully cultured from target tissue over a period of time.[20,34]
Proof of Latency	Reactivation and the production of infectious progeny.[15,34]
Infectious HSV-1 (as it relates to latency)	Infectious HSV-1 cannot be present during latency by definition.[15,34]
HSV-1 DNA (as it relates to latency)	HSV-1 DNA must be present in latent tissue and usually takes the form of circularized episomes or concatamers, or both.[15,34]
LAT (as it relates to latency)	Although LAT is not required to establish latency, it is thought to enhance the establishment of latency perhaps through inhibiting apoptosis. LAT has been suggested to facilitate viral reactivation.[24,33]
Neuronal Latency	Ability of virus to occupy a host neural cell without producing virions for an extended period of time. Virus enters a ganglionic neuron during primary infection and travels in a retrograde fashion to the nucleus, where it lies dormant until stimulation, when the latent virus reactivates, replicates, and produces the viral proteins necessary to assemble infectious HSV-1 virions.[15]
Proof of Corneal Latency	Four requirements must be met in a single cornea **1** Presence of intact genome capable of reactivation and production of infectious progeny **2** Absence of intact virions on electron microscopy **3** LAT is the only transcript abundantly produced **4** No HSV-1 proteins expressed.[15]
Asymptomatic HSV-1 Shedding (Infectious HSV-1 and/or HSV-1 DNA)	Presence of HSV-1 in bodily fluids and skin and most likely represents reactivation from neuronal sites of latency.[33]
Infectious HSV-1 (as it relates to shedding)	Infectious virus is shed through skin or bodily fluids in an asymptomatic individual. Responsible for majority of transmission and recurrence.[6,33]
HSV-1 DNA (as it relates to shedding)	Genetic material shed through bodily fluids of an asymptomatic individual.[6,33]

Table 2

HSV-1 Detected in Mice (Selected Examples)

Author/Year/ (Ref No.)	Groups/ Conditions	Tissue	Virus	Virus component	No. positive/Total no. (%)	Detection Method
Hill et al./1979/(41)	Ear stimulated	Ear	HSV-1 (SC-16)	Infectious HSV-1	9/31 (29)	Culture
	Ear not stimulated				7/88 (8)	
Easty et al./1987/(3)	Inoculated in Cornea	Anterior segment	HSV-1 (SC-16)	Infectious HSV-1	0/48(0)	Culture
	Inoculated in snout				8/20 (40)	
Abghari et al./1988/(18)	Strain DBA/2	Whole eye	HSV-1 (STU-4)	Infectious HSV-1	2/10 (20)	Culture
	Strain BALB/c				2/12 (17)	
	Strain C57BL/6				2/28 (7)	
	Strain DBA/2	Cornea	HSV-1 (STU-4)	Infectious HSV-1	3/17 (18)	
	Strain BALB/c				2/16 (12)	
	Strain C57BL/6				0/17 (0)	
Abghari et al./1992/(19)	Each pool/specimen contains 2 corneas	Cornea	HSV-1 (STU-4)	LAT	5/20 (25)	In situ hybridization
Sawtell /1997/(44)	Corneal inoculation	Trigeminal ganglia	HSV-1 (17Syn+)	HSV-1 DNA	37/37 (100)[∞]	PCR
Sawtell et al./ 1998/(43)	Corneal inoculation Hyperthermia induction	Tears	17Syn+	Infectious HSV-1	19/24 (79)	Culture
			KOS/M	Infectious HSV-1	14/53 (26)	Culture
			KOS/1	Infectious HSV-1	2/13 (15)	Culture
			17/1	Infectious HSV-1	10/12 (83)	Culture

[∞] Sawtell[44] reported the percent of neurons positive in TGs was ~7.35% (range 1–30%) and the copy numbers of HSV-1 DNA per neuron ranged from 10 to 1,000.

[*] Sawtell et al.[43] reported the low phenotypic reactivator of HSV-1 strain KOS/M compared to 17Syn+, a high phenotypic reactivator.

Table 3

HSV-1 Detected in Rabbits (Selected Examples)

Author/Year/(Ref. No.)	Groups/Conditions	Tissue	HSV-1 Strain	Virus Component	No. positive/ No. total (%)	Detection Method
O'Brien and Taylor/1989/(20)	Operational Definition of Latency	Cornea	RE	Infectious HSV-1	10/88(11)	Culture
		TG			68/68(100)	
Zheng et al./1999/(16)	Retrograde migration from latent cornea to naïve host Induction: iontophoresis	Corneal rim	17Pr (17Syn+)*	HSV-1 DNA	3/11 (27)	DNA PCR
		TG			2/11 (18)	
		Tears (eyes)			4/6 (67)	
Cook et al./ 1986/(21)	Operational Definition of Latency	Corneal Epithelium	Glasgow 17 (17Syn+)	Infectious HSV-1	18/24 (75)	Culture
		Corneal Keratocyte			14/24 (58)	
		Corneal Endothelium			16/24 (66)	
Cook et al./1987/(22)	Operational Definition of Latency	Cornea	HG-52, 17, McKrae, R40/2	Infectious HSV-1	4/33(12)	Culture
		TG			33/33(100)	
Sabbaga et al./1988/(23)		Cornea	RE	Infectious HSV-1	26/26(100)	In-situ hybridization
Cook et al./1991/(24)		Cornea	17Syn$^+$, McKrae	HSV-1 TK/ ICP-0 DNA	3/5(60)	DNA PCR
		TG			4/4(100)	
		Cornea		HSV-1 LAT	2/22(9)	RNA PCR
		TG			22/22(100)	
O'Brien et al./1998/(25)		Cornea	RE	HSV-1 DNA	17/30(57)	DNA PCR
		TG			30/30(100)	
		Cornea		HSV-1 LAT	0/17(0)	RNA PCR
		TG			30/30(100)	
		Cornea		HSV-1 TK RNA	10/17(59)	
		TG			23/30(76)	
Openshaw et al./1995/(11)	None showed reactivation	Transplanted Corneas	McKrae	HSV-1 DNA	5/5(100)	DNA PCR

*17Pr is the LAT positive rescue of 17ΔPst. The parent was 17Syn+ and LAT positive.

Table 4

HSV-1 Detected in Non-Human Primates (Selected Examples)

Author	Group/Condition	Tissue	Virus Component	No. Positive/No. Total (%)	Detection Method
Varnell et/ al./1987/(45)	Squirrel monkeys/virus detected at recurrence	Tear Film	Infectious HSV-1 Rodanus	2/14 (14)	Culture
Rootman et al./1990/ (46)	Squirrel monkeys/virus detected after stimulation with 6-HD and EPI	Tear film	Infectious HSV-1 McKrae	8/10 (80)	Culture
	Squirrel monkeys/virus detected after stimulation with timolol	Tear film	Infectious HSV-1 McKrae	7/8 (88)	Culture

Table 5

HSV-1 Detected in Human Corneas (Selected Examples)

Author/Year/(Ref no.)	Group/Condition	Virus Component	No. Positive/ No. Total (%)	Detection Method
Remeijer et al./2001/(12)	Graft to host transmission	Infectious HSV-1	1/1 (100)	Clinical/PCR
Thuret et al./2004/(13)	Graft to host transmission	Infectious HSV-1	1/1 (100)	Clinical/PCR
Biswas et al./2000/(26)	Failed transplanted grafts	Infectious HSV-1	2/2 (100)	Culture, PCR, IHC, TEM
Rezende et al.*/2004/(47)	New onset HK** after PKP†	Infectious HSV-1	14/14 (100)	Clinical
Remeijer et al./2009/(48)	PKP pts‡ with HK	HSV-1 DNA	40/83 (48)	PCR
	PKP pts unrelated to HK		15/367 (4)	
Tullo et al./1985/(1)	Known HK pts	Infectious HSV-1	6/9 (66)	Culture
Shimeld et al./1982/(2)	Known HK pts	Infectious HSV-1	2/3 (66)	Culture
Coupes et al./1986/(4)	PKP pts with asymptomatic HK at surgery	Infectious HSV-1	2/8 (25)	Culture
Easty et al./1987/(3)	Known HK pts	Infectious HSV-1	10/34 (29)	Culture
Laycock et al./1993/(53)	Quiescent corneas after stromal keratitis	HSV-1 DNA	7/13	PCR
		HSV-1 RNA transcripts (3 classes + LAT)	0/13	In situ hybridization
Cantin et al./1991/(5)	PKP pts unrelated to HK	HSV-1 TK DNA	8/11 (72)	PCR
	PKP pts for HK		4/11 (36)	
Openshaw et al./1995/(11)	Eye bank corneas	HSV-1 DNA	9/24 (38)	PCR
Robert et al./2003/(27)	PKP pts unrelated to HK	HSV-1 DNA	8/38 (21)	PCR
Van Gelderen et al./2000/(28)	PKP pts unrelated to HK	HSV-1 TK DNA	13/78 (17)	PCR
Kaye et al./1991/(29)	PKP pts unrelated to HK	HSV-1 DNA	5/10 (50)	DNA PCR
		LAT RNA	3/10 (30)	RNA PCR

** HK – herpetic keratitis

† PKP – penetrating keratoplasty

‡ Pts – patients