Published in final edited form as:

Future Microbiol. 2011 August ; 6(8): 877–907. doi:10.2217/fmb.11.73.

Ocular herpes simplex virus: how are latency, reactivation, recurrent disease and therapy interrelated?

Lena J Al-Dujaili¹, Patrick P Clerkin¹, Christian Clement¹, Harris E McFerrin², Partha S Bhattacharjee^{1,2}, Emily D Varnell¹, Herbert E Kaufman^{1,3,4}, and James M Hill^{†,1,3,4,5}

¹Department of Ophthalmology, Louisiana State University Health Sciences Center, New Orleans, LA, USA

²Xavier University of Louisiana, LA, USA

³Department of Pharmacology, Louisiana State University Health Sciences Center, New Orleans, LA, USA

⁴Department of Microbiology, Louisiana State University Health Sciences Center, New Orleans, LA, USA

⁵Neuroscience Center, Louisiana State University Health Sciences Center, New Orleans, LA, USA

Abstract

Most humans are infected with herpes simplex virus (HSV) type 1 in early childhood and remain latently infected throughout life. While most individuals have mild or no symptoms, some will develop destructive HSV keratitis. Ocular infection with HSV-1 and its associated sequelae account for the majority of corneal blindness in industrialized nations. Neuronal latency in the peripheral ganglia is established when transcription of the viral genome is repressed (silenced) except for the latency-associated transcripts and microRNAs. The functions of latency-associated transcripts have been investigated since 1987. Roles have been suggested relating to reactivation, establishment of latency, neuronal protection, antiapoptosis, apoptosis, virulence and asymptomatic shedding. Here, we review HSV-1 latent infections, reactivation, recurrent disease and antiviral therapies for the ocular HSV diseases.

Keywords

antivirals; chemotherapy; herpes simplex virus; keratitis; latency; ocular; reactivation

History & classification

Herpes simplex virus (HSV) is one of the most common viruses acquired by humans [1]. Accounts of HSV infection date back thousands of years and we are still afflicted today by this virus that is found worldwide. HSV-1 belongs to the family *Herpesviridae* and sub-family *Alphaherpesvirinae* along with HSV-2 and varicella zoster virus (VZV). All three

© 2011 Future Medicine Ltd

[†]Author for correspondence: Tel.: +1 504 568 2274 ■ Fax: +1 504 568 2385 ■ jhill@lsuhsc.edu.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

viruses are neurotrophic and have the unique ability to remain latent in sensory and autonomic ganglia, innervating the site of primary infection for the lifetime of the host [2]. This article emphasizes the most recent literature with respect to HSV-1 latency and reactivation, as well as treatment of herpes keratitis (HK).

Significance

Herpes simplex virus type 1 is a ubiquitous virus that is capable of causing a wide spectrum of human diseases, including herpes labialis (cold sores), gingivostomatitis, herpetic whitlow, genital herpes, epithelial and/or stromal keratitis and encephalitis [2]. Herpetic eye disease can manifest initially as a blepharitis, conjunctivitis or corneal epithelial keratitis [3]. Recurrent herpetic eye disease manifests predominantly as an ulcerative and/or stromal keratitis. Recurrent ocular infections account for the majority of visual morbidity due to irreversible corneal scarring, thinning, neovascularization and eventual blindness [3,4]. In fact, recurrent ocular HSV-1 is the leading cause of infectious corneal blindness in industrialized nations [4].

Epidemiology

Most humans are infected with HSV-1 during childhood or early adolescence via nonsexual contact. At least 90% of the world's population is latent with HSV-1. By the age of 60, more than 90% of the population is latently infected with HSV, as evidenced by immunological data and the high percentage of subjects with HSV-1 DNA in their trigeminal ganglia (TG) [5–14]; yet, a review by Xu *et al.* [1] showed that the overall seroprevalence of HSV-1 in the USA has decreased by 7%, based on surveys carried out in 1988–1994 and 1999–2004. The authors hypothesize that this decrease could be attributed to improvements in living conditions and better hygiene. Nevertheless, herpetic eye infection remains a serious clinical problem, capable of causing devastating ocular diseases and loss of sight [15].

Manifestations

Holland and Schwartz [16] defined four major categories of HSV keratitis: infectious epithelial keratitis consists of corneal vesicles, dendritic ulcers, geographic ulcers and marginal ulcers. These lesions result from active viral replication within the epithelium with patients reporting pain, photophobia and slight watery discharge. Dendritic ulcers are the most common presentation of HSV keratitis. The second category, neurotrophic keratopathy, includes punctuate epithelial erosions and neurotrophic ulcers. These entities have a multifactorial etiology and are neither primarily infectious nor immunologic [15,16]. Neurotrophic retinopathy often results after a long history of dendritic ulcers and treatment with multiple antiviral agents, cumulating in the innervation to the cornea being damaged and diminished tear production [17]. The third category is stromal keratitis, which can be divided into necrotizing stromal keratitis (due to replicating virus) and immune stromal keratitis (due to an immune reaction) [15,16]. A total of 20–60% of patients with recurrent or chronic ocular herpes have stromal keratitis [18–20]. In necrotizing stromal keratitis, there is no defect in the epithelium but the stroma has dense infiltration, ulceration and necrosis owing to direct viral invasion of the stroma [15,16]. Immune stromal keratitis is the more common of the two forms of stromal keratitis and results from recurrent and chronic HSV infection of the corneal stroma. It is thought to be the result of an inflammatory response from CD4⁺ T cells stimulated against HSV-1 antigens or auto-antigens mimicked by HSV-1, to bystander cytokine activation [17]. The fourth category of keratitis, endotheliitis (due to an active infection), is primarily an inflammatory response to the endothelium. Endotheliitis can be further subdivided into disciform, diffuse and linear types [15,16].

Primary infection

Factors influencing acute infection & latency

Viral characteristics—The strain of virus and the dose of inoculum influence susceptibility to acute infection by HSV. Various studies in mouse models found that in acute infection, viral strains differ in terms of pathogenesis and pattern of neurovirulence and retinovirulence, and this may be due to differences in extent and direction of transport transneurally [21,22]. Wander *et al.* studied numerous strains of HSV-1 and determined that different strains presented in various patterns of disease severity in the acute and latent infection [23,24]. A higher number of latent virus copies has been suggested to promote reactivation by overwhelming the cellular mechanisms that silence virus transcription [25]. In guinea pigs, the Shealey strain produced the most severe acute eye infections, characterized by severe epithelial and stromal lesions, corneal neovascularization and ulcerative blepharitis; this strain also produced the highest load of latent viral DNA in the TG [23]. Viral strains such as 17 Syn+, McKrae and KOS/M latently infected similar numbers of mouse neurons, but the number of viral genome copies per neuron was significantly greater in the 17 Syn+ and McKrae strains than in KOS/M [26]. This finding supports the hypothesis that the number of HSV genome copies within individual latently infected neurons is regulated by viral genetic factors, and the latent genome copy numbers may be an important parameter for subsequently induced reactivation *in vivo* [26]. Numerous studies in animal models have reported that the latent viral load within a ganglion is directly related to the frequency or rate of reactivation [26–28]. For acute infection, Centifanto-Fitzgerald *et al.* found that in a rabbit eye model, the duration, incidence and severity of stromal and epithelial disease was directly related to the HSV strain [29]. Their study showed evidence that stromal disease pattern can be transferred to an epithelial disease producing virus by taking specific fragments of the stromal disease producing viral strain. This new recombinant viral strain had disease characteristics of the donor strain. The same was found to be true when the reverse was performed. More recent studies performed by Brandt *et al.* showed that the recombination of different genes from two different viral strains (OD4/CJ394 and OD4/994) produced different outcomes of ocular infection, neurovirulence and mortality [30,31]. They went on to identify certain HSV-1 viral genes, such as U19, -33, -36/37, -42 and US1, which appear to be involved in peripheral virulence [32].

Host characteristics—In addition to the strain of the virus, the genetic make-up of the host and the host's immune responses also influence susceptibility to HSV active infection and reactivation from latency. Differences among inbred strains of mice exert a strong influence on the frequency of HSV-1 reactivation; for example, HSV-1 reactivates much more readily in BALB/c mice than in the C57B1/6 mouse [33,34]. The competence of the host cellular immune response is suggested to be important in controlling reactivation [35,36]. The cornea's outer surface mucin layer and lactoferrin, an antiviral molecule present in tear film, could help prevent initial active HSV-1 infection [37]. Furthermore, other host cell factors such as nitric oxide, TNF and interferon have been suggested to affect the severity of the active HSV infection [38–40]. A genetic locus linked closely to TNF p55, discovered by Lundberg *et al.* [40], named herpes resistance locus (Hrl), was found to determine susceptibility or resistance to HSV-1. Furthermore, Jouanguy *et al.* [41] suggested that humans who are genetically deficient in their capacity to either produce or respond to type 1 interferon are subject to more severe HSV active infections. A recent study found that type 1 interferon regulatory factor-3 (IRF-3) is needed for timely and effective response to HSV-1. IRF-3^{-/-} mice were more susceptible to intracranial and corneal infections due to HSV-1 [42]. For the latent infection, Hoshino *et al.* reported in a mouse model that the

reactivation of HSV-2 from ganglia *ex vivo* is influenced by the number of infiltrating CD8⁺ T cells [43].

Animal studies and limited observations in humans suggest that a substantial risk of susceptibility to herpetic diseases is inherited. Lopez suggested that in mice, the major histocompatibility (MHC) locus, in particular gene H-2, does not play a role in susceptibility or resistance to active infection and that non-MHC genes could play a role [38,44]. Critics of this study note that Lopez used intraperitoneal infection so this situation may not be applicable to corneal infections [45]. In a recent study, Hobbs *et al.* found no significant linkage on chromosome 6 in the region known to include the MHC's HLA genes, which suggests that the HLA regions are not a major determinant of the frequency of herpes simplex labialis recurrences [46]. However, other studies did support a role for MHC HLA genes in susceptibility and recurrence of latent infection and have suggested an increased susceptibility to recurrent latent herpes simplex keratitis, labialis and genital herpes [47–49]. Lekstrom-Himes *et al.* reported that the HLA-A1 allele frequency is increased in patients with frequent outbreaks of genital herpes, whereas HLA-B27 appeared to have a protective effect [49]. Similarly, Elner *et al.* reported an increased frequency of the HLA-B5 and Aw30 alleles in patients with HSV keratitis [48].

To help better identify the gene involved in susceptibility to herpes simplex labialis (cold sores or fever blisters), Hobbs *et al.* used genetic linkage analysis to identify a region on the long arm of human chromosome 21 [46]. This region contains six candidate genes for herpes susceptibility and expression of one or more of these candidate genes is likely to affect the frequency of herpes simplex labialis. Of the six genes identified, three were suggested to play a role in the susceptibility of herpes reactivation (**Table 1**).

Recently, Kriesel *et al.* identified C21orf91 as a primary candidate region on the human chromosome for recurrent herpes labialis susceptibility [50]. Kriesel *et al.* further investigated the six possible candidate genes on human chromosome 21 that are thought to be associated with herpes simplex labialis [50]. They reported that different alleles of the human gene *C21orf91* are significantly associated with susceptibility to acute infection. This gene of unknown function appears to be a major determinant of the frequency of labial herpes outbreaks; the authors have proposed that this gene be designated the cold sore susceptibility gene-1 (*CSSGI*) [50].

Another human gene suggested to have a role in increased susceptibility to HSV-1 is the *ApoE* gene, specifically the $\epsilon 4$ allele. Carriers of *ApoE* $\epsilon 4$ have a greater chance of developing ocular disease and cold sores due to an increased risk of reactivation [51–56]. In humans, there are three major allele types: $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$. ApoE $\epsilon 3$ is the most frequently expressed type (77–78%), followed by $\epsilon 4$ (14–15%) and $\epsilon 2$ (7–8%) [57–59]. Studies have suggested that $\epsilon 3$ is anti-inflammatory while $\epsilon 4$ is pro-inflammatory. Currently, research is being conducted to develop a therapeutic agent from apoE that can prevent inflammation and disease progression [60–62].

Mouse models have been used to study the effects of ApoE $\epsilon 4$ and HSV-1. Burgos *et al.* injected the HSV-1 KOS strain intraperitoneally into wild-type (*ApoE*^{+/+}), apoE knockout (*ApoE*^{-/-}) and hemizygote (*ApoE*^{+/-}) mice [51]. They reported the level of *ApoE* gene expression correlated with the invasiveness of HSV-1 in neurons. In reviewing this study, Hill *et al.* concluded that *ApoE*^{+/+} was most susceptible and *ApoE*^{-/-} was the least [55]. In another study, Burgos *et al.* [52] used transgenic mice by replacing the murine *ApoE* with either human *ApoE* $\epsilon 3$ or *ApoE* $\epsilon 4$ and inoculated them with HSV-1. They reported that the $\epsilon 4$ mice had a higher load of HSV-1 DNA in ventricles, cortex, cerebellum and midbrain than $\epsilon 3$. Interestingly, $\epsilon 3$ viral load was low or undetectable. In a review, Hill *et al.* proposed

that this was direct evidence that $\epsilon 4$ increased the neuroinvasiveness of HSV-1, susceptibility and severity compared with $\epsilon 3$ [55]. Further evidence suggests that ApoE $\epsilon 4$ aids in the establishment of HSV-1 latency more so than $\epsilon 3$, leading to higher viral loads [53,63]. Finally, Bhattacharjee *et al.* investigated whether ApoE $\epsilon 4$ has a role in ocular herpes using a mouse model. Mice with either knock-in ApoE $\epsilon 3$ or $\epsilon 4$ and the parent, C57B1/6, were inoculated via the cornea with HSV-1 [56]. They reported that both the apoE $\epsilon 4$ mice had significantly more HSV-1 DNA than $\epsilon 3$ mice, indicating that murine ApoE exhibits similar effects on neuroinvasiveness compared with human ApoE, as was previously shown [52,53,63]. An earlier onset of corneal opacity, increased viral replication in the cornea, increased incidence of and severity of corneal neovascularization and increased viral load in the brain and TG were found in ApoE $\epsilon 4$ mice compared with ApoE $\epsilon 3$. Furthermore, $\epsilon 4$ mice exhibited upregulated expression of mouse VEGF and proteolysis of ApoE. The lack of mVEGF expression in ApoE $\epsilon 3$ mice could be a possible explanation of the decreased neovascularization in ApoE $\epsilon 3$ mice. These two factors were suggested to contribute to the increased pathogenesis of ApoE $\epsilon 4$ [56]. To our knowledge, few human studies have been conducted to answer this question. Lin *et al.* suggested ApoE $\epsilon 4$ as a risk factor for herpes labialis after noting a higher rate of this allele in subjects with herpes labialis compared with age-matched subjects [54]. A clinical study evaluating herpes keratitis in 46 patients found no correlation between the frequency of ApoE $\epsilon 4$ and HK [64]. The 46 subjects with HK had a 15% (7/46) frequency of the $\epsilon 4$ allele; 238 disease-free subjects had the same percentage (15%) of the ApoE $\epsilon 4$ allele. One concern for this study was the small sample size and insufficient details of the clinical characterization and description of the herpes ocular infection. More studies are needed to assess the role of ApoE in terms of acute infection (severity), latency and reactivation of HSV-1 as well as frequency.

Environmental factors

Another variable influencing susceptibility to infection is the environment. Social stress, hypothermia, hyperthermia, skin irritation and exposure to UV light are known triggers for HSV reactivation in animal models [2,65,66]. In humans, fever, wind, sunburn, trauma and surgical manipulation of the trigeminal ganglion have been shown to induce HSV reactivation [2,66–68].

Lytic phase genes

Immediately after entering the host cell, HSV-1 shuts off host cell protein synthesis via the virion host shutoff (vhs) protein. This protein, which allows the degradation of host mRNA early in infection, is blocked late in the infection by a regulatory protein, VP16, to prevent the breakdown of viral mRNA [69]. Viral DNA is then released into the nucleus via a nuclear entry pore and host RNA polymerase II transcribes the viral genes [70,71]. Within 2–4 h post infection, viral protein synthesis begins. Gene expression occurs in three distinct phases, immediate early (IE), early (E) and late (L) [2,72].

Immediate early genes are important in the expression of their own genes via feedback mechanisms. The IE genes include *ICP0*, *ICP4*, *ICP22*, *ICP27* and *ICP47* [2,72]. *ICP4* upregulates the E and L genes and downregulates the IE genes through interaction with the transcription factors associated with RNA polymerase II. In addition, *ICP4* represses the latency-associated transport (LAT) promoter and prevents LAT production during acute infection [73–77]. Also, as with *ICP0*, *ICP4* is suggested to block the initiation of apoptosis [78–80]. *ICP0* activates viral transcription and promotes viral replication through interactions with chromatin-remodeling enzymes without directly binding DNA [81]. During productive infection, *ICP0* has been shown to stimulate viral gene expression by blocking histone deacetylation (HDAC) and preventing the circularization of the viral

genome [82,83]. ICP0 has been suggested to have the same function during reactivation from latency and may be the molecular switch [84]. ICP47 prevents the transport of viral antigens to the endoplasmic reticulum, thus inhibiting CD8⁺ T cells [85,86].

At 5–7 h post-infection, E genes peak [2]. These genes are important for HSV-1 replication and include ICP6, ICP8 and viral thymidine kinase (TK). ICP6 is a subunit of ribonucleotide reductase [66,87] and ICP8 is a DNA binding protein [2]. HSV TK has the unique ability to phosphorylate pyrimidine and purine nucleoside analogs that the host cannot. Nucleoside analogs, such as acyclovir (ACV), are used in the treatment of herpetic infections and rely on viral TK for activation of antiherpetics for inhibition of viral replication [3,88,89]. Finally, after viral replication has peaked, the L genes peak [2]. The gene products are tegument components, structural components, capsid-associated components and glycoproteins [2]. In most cell cultures, the virus is assembled and released with the entire replication process taking approximately 12 h [2]. *In vivo*, HSV-1 then moves from cell to cell and eventually egresses using cellular transport mechanisms [90,91]. In animal models that support HSV infection, gene expression and replication are detected for approximately 5–14 days in TG before latency is established [89,92].

Latency

Definition

Latency is the ability of HSV to retain at least one functional viral genome in a host cell nucleus in the absence of virus replication, assembly or pathological effect. In this state, the 152 kb DNA genome is maintained as multiple copies of circular episomes as opposed to the linear form found during lytic state [2,35,87]. Alphaherpesvirus is characterized by establishing latency in neuronal cells [93,94]. Divito *et al.* suggested that the maintenance and regulation of latency in HSV-1 involves a tripartite relationship between neuron, virus and CD8⁺ T cells [84]. In addition, recent evidence shows that latency could be restricted to a certain subtype of neuron, one that is recognized by marker antibody A5. This neuron-specific protein may interact with elements of the HSV genome, in particular LAT [87]; however, the exact mechanism of how latency is established and maintained is not known [66,87,94].

Latency occurs as part of a three-step cycle: establishment, maintenance and reactivation [2,66,94]. Establishment occurs during active infection when the virus replicates within the mucosal epithelial cell and enters its sensory neuron, then travels to the sensory ganglia in a retrograde fashion via the cell's microtubule network. Since most HSV-1 infections occur via the ocular, nasal or oral sites, they can be latent in the sensory neurons of the TG [87,90,91]. Latency is established when the viral genome is transcriptionally repressed except for LAT and miRNAs [66,94]. Although some studies have suggested that LAT has no role in latency, the majority of the studies concluded that LAT plays an important role in latency, but is not required for establishment of latency [66,84,90,91,94–96]. Studies in murine and rabbit models have suggested that LAT enhances the establishment of latency [97–99]. The possible functions of LAT are discussed further in the next section.

The second phase, maintenance, lasts the lifetime of the host. During this phase, only the expression of LAT is observed abundantly with all other gene expression generally repressed [94]. Small miRNAs are also present in latent neurons. The term 'operational latency' applies at this time. Two criteria must be met for operational latency: the infectious virus cannot be present in a neuron or detected by standard isolation procedure, plus the infectious virus must be successfully obtained from explanted target tissue (i.e., TG) [100–102]. The third phase, reactivation, can be spontaneous (no known inducer) or induced by an external stimuli such as fever, psychological stress, fatigue, hypothermia, hyperthermia,

immunosuppression and UV-B exposure [65,66,94]. This multistep process results in stimulation of viral gene expression. HSV gene expression (mRNA and proteins) can be detected in sensory neurons and infectious HSV can be isolated from the TG, saliva and tears [66,94]. The viral particle is transported by an anterograde flow to the periphery [103,104]. The end result is virus transmission and recurrent disease [66,84].

Functions of LAT

The 152,261 bp of the HSV-1 genome are divided into two covalently linked regions, the unique long and unique short. Both unique long and unique short are flanked by inverted repeat sequences called inverted repeat short and inverted repeat long [2]. Coded within the inverted repeat long is the 5' end of *LAT* gene [105]. During establishment, up to two-thirds of the neurons in the ganglia can express high quantities of LAT, mainly in the form of the 8.3–8.5 kb polyadenylated transcript [2,107]. The transcript is then further spliced to yield 2.0, 1.5 and 1.45 kb very stable mRNA introns [106–108]. The high stability is due to the intron being in a lariat form versus a linear molecule [109,110]. The promoter of LAT, LAT-associated promoter (LAT-1), is activated in sensory neurons and contains an enhancer element that is important in the expression of LAT during latency [108,111]. This enhancer element, named long-term expression, maintains LAT promoter activity and LAT expression during long latency periods [108,112,113]. Although LAT has many open reading frames (ORFs), is polyadenylated and capped, most studies provide no evidence that it encodes any proteins [2,66,114]. However, Naito *et al.* identified a gene upstream of LAT that encodes a putative protein with predictive mass of 11 kb [115]. The function of the protein is not known, but the RNA of this protein is expressed in infected cells as an L gene product. Furthermore, several other studies have reported that a protein is encoded and expressed within the first 1.5 kb region of LAT [116–121]. Various functions of the protein have been suggested. Thomas *et al.* suggested that the LAT protein could substitute for ICP0 due to its similar structure [116,117]. Randall *et al.* suggested that the protein could have a role in preventing ICP4 from binding to its cognate site on DNA [118]. Even if LAT encodes a protein, the role of the protein still remains unclear, as does its role in the latency-reactivation cycle of HSV-1 [66,94]. In addition, if a protein exists, the concentration in HSV-1 latent neurons must be very low.

Latency-associated transcript has several known functions and may contain additional functions that are important for the latency– reactivation cycle. In addition to LATs, this region codes for many other known viral transcripts and miRNAs. In the colinear direction to the 8.5 kb LAT gene, there are two transcripts. One is produced by LAT-associated promoter (LAT-2) and another is the 0.7 kb transcript that extends into LAT-1 [108]. LAT-2 has also been proposed to promote expression of the stable 2 kb intron in culture cells during lytic infection [122–125]. In the antisense direction, there are transcripts called ‘antisense to LAT’ (AL-LAT) AL2 (in the first 1.5 kb), AL3 (originating in the LAT intron) and lytic genes *ICP4*, *ICP0* and *ICP34.5* identified [126,127]. During latency, the other transcripts, including the lytic genes, are repressed. Only the LATs are abundantly transcribed [108]. In addition, in the first 1.5 kb of LAT, two sRNAs were found, called sRNA1 and sRNA2 [128]. Within the LAT promoter, two cAMP-response elements (CRE) were found to be involved in inducing reactivation via second messenger pathways involving cAMP [129–133]. Both Bloom *et al.* [129] and Marquart *et al.* [133] studied the effects of HSV viral recombinants with CRE site-directed mutation in both rabbit and mouse ocular models, respectively. Both found that CRE locus is needed for efficient reactivation; those animals infected with a CRE recombinant HSV-1 exhibited a reduction in both spontaneous and induced HSV-1 reactivation [129,133].

The LAT genome region encodes for miRNAs. These are noncoding sRNAs that miRNAs have the ability to bind to a target mRNA and regulate its expression [134–137]. Owing to

this ability, their nonimmunogenic nature and small size, miRNAs are an ideal regulatory molecule. There are seven miRNAs in the primary LAT transcript and two are located in the promoter [134,136]. Umbach *et al.* reported that LAT miRNAs, miR-H6 and miR-H2-3p, reduce *ICP0* and *ICP4* expression, respectively [134]. Neither of these miRNAs altered the expression for miRNA that encoded *ICP4* and *ICP0* [134]. The suppression of these two important lytic genes suggests that viral latency may be maintained and established by LAT's miRNAs. The Umbach *et al.* study was carried out *in vitro* [134]. Currently, there are no data showing the effects on viral replication *in vitro* when the miRNAs are deleted from the viral genome, or how the miRNAs could alter the pathobiology of the virus *in vivo* [108].

Latency-associated transcript has many potential (suggested) functions. These include HSV reactivation, establishment of latency, survival/ virulence, antiapoptosis, neuronal protection and virulence/apoptosis [108]. Animal models are employed to study latency because a true latent state is hard to achieve *in vitro*. The two most common animal models used are the mouse and rabbit eye models.

The mouse model is by far the most common model for the investigation of HSV-1 latency, in part owing to many available genetically constructed strains (knock-out/in) and the cost [66]. **Box 1** lists advantages and disadvantages of the mouse eye models of HSV-1 acute, latent and recurrent disease. Infection of the mouse can occur on the eyes, flank, ear pinnae, snout or footpad. Following HSV infection, the virus stays latent indefinitely in mouse neurons. Reactivation can be achieved *in vitro* by removing the TG and placing them in culture media. This method is called 'explantation-induced reactivation' [138,139]. Disadvantages of the mouse model include the small amount of tissue provided and the small size of the mouse eye, making slit-lamp examination difficult [66]. Latency in the rabbit model can be established in TG following corneal infection. The rabbit eye model has the advantage of the potential ability to produce clinical reactivation at the primary site of infection [87]. **Box 2** lists the advantages and disadvantages of the rabbit eye models in HSV-1 acute, latent and recurrent disease.

Typical reactivating stimulators used include adrenergic agents such as epinephrine and timolol, applied either topically or by iontophoresis [140,141]. Epinephrine is most commonly used due to its high frequency of reactivation (approaching 100%) and long duration (typically 3–5 days) [66]. Other advantages over the mouse model are that more tissue can be obtained and that the eye size of the rabbit is more similar to humans, allowing ease of slit lamp examination [66]. Finally, certain HSV-1 strains such as McKrae and 17 Syn+ have been found in various studies [26,142,143] to exhibit a high rate of spontaneous shedding in comparison with other strains, such as KOS.

The phenotypic activities attributed to LAT will be reviewed here.

Reactivation—Reactivation was one of the first phenotypes attributed to LAT. In an early study, Hill *et al.* found that in the HSV-1 intertypic recombinant X10–13, which lacked LAT, the reactivation and subsequent HSV-1 shedding were much less frequent than in the LAT-positive recusant strain, X10–20 [144]. *In vitro* studies with mouse explants with HSV strain Δ1LAT1.8 (which lacks the promoter, start site and 1015 bp of the LAT DNA sequence) reported that reactivation was 49% less effective compared with the parent strain and the time for infectious virus to appear was longer [145]. Thus this region (1.5 kb) appears to be a critical factor for *in vitro* reactivation [146]. Bloom found that a small deletion within the 5' exon region of LAT had no effect on reactivation compared with a large deletion (> ~350bp) [108]. Furthermore, whether or not the 2.0 kb intron of LAT was present, reactivation could occur. Overall, various studies in mouse and rabbit eye animal

models show that LAT is not absolutely necessary for reactivation; however, LAT negative strains showed a significant decrease in the *in vivo* reactivation rates [66,108,144,147].

Establishment of latency—Studies by Steiner *et al.* [148] and Izumi *et al.* [95] demonstrated that LAT expression is not necessary for establishment or maintenance of latency. These two studies took HSV-1 viral strains (1704 and KOS 8117, respectively) lacking LAT expression and compared them with the parent strains. Both experiments found HSV latency in both mutant and wild-type strains after explantation of ganglia in cell cultures [95,148]. However, in a recent review article, Perng and Jones suggested that although LAT is not necessary, it might play a role in establishing latency [94]. Studies in both murine and rabbit models suggest that LAT enhances the establishment of latency and increases reactivation from latency [97,98,99,146]. Thompson and Sawtell performed a study using mouse models with either wild-type HSV-1 virus (KOS/M) or mutant strains (KOS/29 and KOS/62) [97]. KOS/29 lacked the LAT promoter and KOS/62 lacked 1.6 kb from the 5' end of LAT. Mice infected with KOS/M had 30% of their neurons latent with HSV-1 while those infected with either mutant strain had only 10%. Furthermore, latency was restored when mutant strains were repaired or expression of the 2.3 kb fragments that contain the major LAT sequences (2.0 kb and 1.5 kb introns) was restored. The authors suggest that the 2.3 kb fragment is the region responsible for establishing the latency phenotype. This fragment encodes the stable LAT introns (2.0 and 1.5 kb) [97,149].

Thompson and Sawtell analyzed murine TG for HSV-1 DNA using well-controlled quantitative PCR [149]. The 17 Syn+ HSV-1 viral strain (wild-type) and 17AH (LAT-negative mutant) were used. They found a 75% reduction in the amount of HSV-1 DNA in the murine TG of mice infected with the 17AH compared with the wild-type and rescue strain [149]. The authors suggested that LAT increases the number of latent neurons and promotes neuronal survival by the ability of LAT to downregulate lytic genes and inhibit viral replication in neurons [149].

Finally, in rabbit eye models, Perng *et al.* inserted a gene, enhanced green fluorescent protein (EGFP), under control of the LAT promoter into both LAT-positive and LAT-negative strains [99]. TG of the rabbits were examined following inoculation with EGFP-expressing virus. More neurons containing the EGFP were found in the eyes infected with LAT-positive strains than those infected with LAT negative strains [99]. Bloom suggested that these studies demonstrate that LAT enhances latency by increasing the amount of HSV-DNA present and the number of cells that are latent [107].

Summary of establishment of latency—Two reviews by Roizman [150] and Kristie *et al.* [151] have summarized the regulation of productive infection and the establishment of latency highlighting host and viral factors. Kristie *et al.* have focused primarily on host control factor (HCF) 1 and has provided evidence of how HCF1 coupled with chromatin-modifying activities regulated HSV-1 IE expression [151]. Roizman has proposed four checkpoints for HSV-1 gene expression in productive and latent infections [150]. The checkpoints involved the following factors: histone deacetylase inhibitor, lysine specific deaminase 1 (LSD-1), receptor element-1 silencing transcription (REST) and co-repressor of REST (Co-REST). Roizman noted in his conclusions that the repressor complex involving neuronal reactivation is supported by Neumann *et al.*, who has shown that an histone deacetylase inhibitor can induce HSV-1 reactivation in mice [152]. In addition, Roizman noted an expectation that other potential host cell pathways could be exploited by HSV-1 and that some of the hypotheses presented could be a challenge [150].

Neuronal protection (survival)—Herpes simplex virus type 1 survives by establishing latency and not killing the host cells. Death of the neurons in the TG may occur after

frequent reactivation and entry of HSV-1 into the lytic phase [108,149]. The Thompson and Sawtell study proposed that LAT has a role in establishing latency by downregulating lytic gene expression, inhibiting viral replication in the neurons and, in the process, preventing neuronal death [149]. They found that the 17AH strain had twice the neuron loss compared with 17 Syn+. Furthermore, the neuronal death did not seem to be attributed to increased apoptosis based on measures by a terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) assay. The loss of these neurons is a possible explanation for reduced establishment of latency in the LAT mutants [108].

Antiapoptosis—Latency-associated transcript has been reported to play a role in inhibiting apoptosis. In experiments using TG of mice and rabbits, non-LAT-expressing HSV-1 induced increased apoptosis compared with LAT-expressing HSV-1, evidenced by the TUNEL assay. In addition, this experiment found an increase in p85 cleavage products of poly-ADP-ribose-polymerase in non-LAT strains [153]. This finding suggested that LAT encodes a functional product, possibly a protein, which results in inhibition of caspase 3 [108,153]. Furthermore, plasmids expressing LAT interfered with the apoptosis pathway of sympathetic and sensory neurons deprived of nerve growth factor by inhibiting the activation of caspase 3 and decreasing the percentage of neurons exhibiting DNA condensation [154]. Another experiment studied this antiapoptotic effect on both the TG and CD8⁺ T-cells recruited to the infected neurons. The TG of mice inoculated with a LAT-negative mutant had significantly more apoptotic neurons than the TG of mice infected with the parent strain and significantly more CD8⁺ T cells underwent apoptosis in the LAT-negative mutant [155]. Plasmids expressing LAT were reported to inhibit apoptosis by inhibiting caspase 8 and 9 [156,157]. One experiment showed that the 2 kb LAT intron, found in both the nucleus and the cytoplasm, has an affinity for the ribosomal and splicing complexes in HSV-1 infected cells. This association is hypothesized to affect the apoptosis pathway by inhibiting apoptotic proteins or aiding in the production of antiapoptotic factors [158]. Experiments that introduced ATG-TTG mutations in six ORFs within the 1.5 kb intron were found to impair the antiapoptotic function of LAT [159]. This suggests that LAT alters RNA structure or encodes a functional protein. Recently, the same mutations were introduced into the two sRNAs located within the 1.5 kb intron. Not only did this inhibit their synthesis, but it also inhibited the antiapoptotic function, suggesting that the two sRNAs coded in the 1.5 kb intron have a role in preventing apoptosis [136].

Virulence & apoptosis—Studies have also shown that deletions in LAT can alter virulence and paradoxically induce apoptosis. Certain regions within the LAT domain seem to alter virulence when deleted. In some models, the alteration was reported to increase virulence, owing to an increased viral replication and cell death in the target tissue [160]. In other situations, there is only a slight decrease in virulence and infectious viral production occurred [161]. None of these effects has been noted in cell cultures; it seems to be an *in vivo* phenomenon [108]. In the book *Alpha Herpesviruses: Molecular and Cellular Biology* in a chapter by Bloom, he proposed that the paradoxical apoptosis function of LAT could be an RNA antisense to the 5' end of LAT named AL-RNA [108]. Perng *et al.* suggested this might play a role in viral virulence and suggested that it could encode a 56-amino acid protein [126]. The role of the protein encoded by AL-RNA with apoptosis has not been established.

Asymptomatic shedding—Between the ages of 65 and 70, almost 100% of the population is seropositive for HSV-1 [1,2,66,93,162]. A recent study by Hill *et al.* evaluated human TG for HSV-1 DNA using real-time PCR from a wide age range (birth to >90 years) in both males and females [14]. A total of 89% of the human subjects were found to be positive for HSV-1 DNA in their TG, with no association to age or gender. The high

frequency of HSV-1 DNA shedding seems to be from asymptomatic individuals [93,163,164]. Asymptomatic shedding is the presence of HSV without any clinical symptoms [165]. Since HSV-1 is able to establish latency for the lifetime of the host, many healthy individuals apparently shed the virus without having symptoms. In fact, clinically, only 1–3% of primary ocular infections are recognized. Hence, most transmission of HSV-1 is due to asymptomatic shedding [163]. Mucosal surfaces, such as the mouth [165], eyes [163,166], skin [167] and genitalia [168] tend to be common sites of HSV-1 shedding [93,163,165].

Before reviewing past and recent studies of asymptomatic shedding, it is relevant to discuss the different detection techniques. Quantitative PCR (qPCR) is the most sensitive test available to detect HSV-1 DNA and is the current gold standard with a specificity of 78% and a sensitivity of 82% [169,170]. Compared with antibody serology assays, PCR is 1000–10,000 more sensitive in detecting HSV-1 [93]. qPCR is not able to detect infectious virus, only HSV-1 DNA. This does not guarantee that infectious particles are present because there is no direct correlation between HSV-1 copy numbers and infectious particles. Furthermore, the detection method is of specific segments; the entire genome may or may not be present [102].

Before PCR became readily available, infectious virus was assayed by cell culture. Cell cultures have a very low sensitivity compared with PCR [167,169,171–173]. An increase of 43.9% in HSV-1 detection was reported in one study when PCR was compared with vial cultures [174]. Higher specificity and sensitivity have been achieved with qPCR [16,163] so that as few as five copies of HSV-1 DNA can be detected.

Various clinical studies have detected infectious HSV in saliva and tears of healthy individuals. The first study that detected HSV-1 in saliva was done by Buddingh *et al.* in 1953 at the LSU School of Medicine [175]. This study of 553 healthy volunteers, children and adults, found that adults had three-times more infectious HSV-1 in their saliva than children. Although the study by Hill *et al.* found no statistical association between age and gender [14], this early study showed that detecting infectious HSV in saliva was correlated to infection being detected at higher rates in adults than children [175]. In 1967, Kaufman *et al.* were the first to report detection of infectious HSV-1 in tears [176]. HSV was detected in the tears of rabbits with latent HSV-1 and in tears and saliva of healthy humans [176].

A recent review cited ten publications on HSV-1 DNA shedding in tears of healthy subjects from 1994–2005 using PCR [93]. This study found a low frequency of HSV-1 detection in tears with the exception of the largescale study done by Kaufman *et al.* [163]. Of the ten studies, five did not detect any HSV-1 DNA. The authors attributed this to a small sampling size; most studies had only 1–2 swabs per subject. Abiko *et al.* had the second largest sampling after Kaufman *et al.* with six swabs per subject, but had low HSV-1 detection at 3.7% [166]. The recent Kaufman *et al.* study was different from the others, collecting tears and saliva samples twice a day for 30 consecutive days in 50 subjects with 60 swabs per subject [163]. Tear samples (~2800) were analyzed by PCR for HSV-1 DNA. The results showed that 33.5% of assayed tear samples (941/2806) contained HSV-1 DNA, equal in men and women but higher in African-American than white subjects. Furthermore, of the 50 subjects, 49 (98%) shed HSV-1 DNA at least once, either in tears or saliva, in the 30 days. Thus, the Kaufman *et al.* study showed an increased shedding in tears, possibly due in part to a higher frequency of samples taken over a longer period of time and the sensitivity of the PCR [163].

Cell culture study of infectious HSV-1 from eye swabs of healthy subjects was the first detection method of asymptomatic shedding in tears. Cohrs *et al.* reviewed five studies

detecting HSV-1 shedding in tears via cell culture [93]. All of these studies had very low detection of infectious HSV-1. Kaye *et al.* found no shedding of infectious HSV-1 although they collected approximately 3 swabs per subjects [169]. Okinaga found 0.06% shedding using approximately 17 swabs per subject, which is considerably less than the recent Kaufman *et al.* study [163,177]. Cell culture and PCR studies cannot be directly compared because cell culture is known to be a less sensitive technique [102].

In comparison to asymptomatic shedding of HSV-1 in tears, saliva has been analyzed more often [163]. Miller and Danaher performed a detailed review of HSV-1 oral shedding, examining results from 1953–2006 [165]. After taking into account sample frequency (i.e., >five times per week) and relying on PCR assays, they found that 70% of people shed HSV-1 in the oral cavity at many intraoral sites. Furthermore, seronegative subjects were also found to shed HSV-1. In the study by Kaufman *et al.*, approximately 2700 oral samples were analyzed quantitatively by PCR for HSV-1 DNA copy numbers [163]. The results showed that 37.5% of assayed saliva samples contained HSV-1 DNA with higher results in men versus women but equal in African–American and white subjects. Of the 50 subjects, 49 (98%) shed HSV-1 at least once in the month in saliva and/or tears; only four subjects did not shed HSV-1 in their tears. Furthermore, 37 of 50 (74%) subjects were IgG positive for HSV-I. Interestingly, Kaufman *et al.* also noted that HSV-1 was shed by a few seronegative subjects [163]. How is that possible? The most likely explanation is that the sensitivity of PCR is at least 1000-times greater than that of the HSV-1 serology assay [14,66,93,163]. Another explanation is that a recent HSV-1 infection was undetected in subjects that had not yet seroconverted [178].

Neuronal latency

Herpes simplex virus-1 establishes neuronal latency, most commonly assessed in the TG [2,16,102,106]. The virus remains latent in the sensory and autonomic ganglia of the head and neck that innervate the primary site of infection. The traditional site for the study of latency is the TG [14,66,179,180,181]. **Table 2** summarizes studies that have detected HSV-1 DNA in human TG. In these studies, HSV-1 DNA was detected in more than 50% of the TGs. Hill *et al.* assayed TG from 174 autopsy samples and found 89% (155/174) had HSV-1 DNA in the TG [14]. The copy numbers ranged from ten copies to approximately 3000 copies per 100 ng of host DNA, indicating that the TG load is highly variable [14]. In addition, no age or gender association was found.

Other sites for latency that have been demonstrated in humans include the superior cervical, spinal, geniculate, vestibular, inferior vagal and ciliary ganglion (CG) [6,180,182]. Reactivation from these nontraditional sites may explain the unusual infections linked to the herpesviruses such as Bell's palsy, Meniere's disease and acute retinal necrosis [2,66]. The CG provides the postganglionic parasympathetic innervations to the anterior chamber. Bustos *et al.* suggested that CG, along with TG and trigeminal nerves, may be involved in the finding that some patients have both herpetic stromal keratitis and anterior uveitis [6]. Furthermore, Bustos *et al.* suggested possible routes of HSV-1 latency in the CG [6]. They suggest the establishment of latency in the CG after initial infection of the anterior chamber and the possibility that HSV-1 is transferred from the TG to CG via the nasociliary path during reactivation.

Richter *et al.* performed a recent experiment investigating all ganglia in the head and neck of human cadavers for HSV-1, VZV and HSV-2 [180]. The ganglia examined were trigeminal, pterygopalatine, ciliary, geniculate, otic, submandibular, superior cervical and nodose ganglia; **Table 3** summarizes the results. Interestingly, the authors did not detect any HSV-2 (0/36 cadavers); however, 89% contained HSV-1 (32/36 cadavers) and 61% (22/36) contained VZV. HSV-1 and VZV were detected in every ganglion of the head and neck.

Furthermore, they found an independent distribution of HSV-1 and VZV. The authors suggested that this independent distribution may explain the unilateral occurrence of HK.

Non-neuronal latency

Corneal latency—The issue of whether HSV-1 can establish nonneuronal latency is an area of great debate. The cornea has been proposed to be a possible site of latency [100,102,183–188]. HSV-1 DNA and infectious DNA have been detected in corneas of mice, rabbits, nonhuman primates and humans latently infected with the virus [183,185,188,189]. However, HSV-1 latency in the cornea has failed to gain full acceptance due to the controversy of whether or not the virus is truly latent in the cornea or only at a transition point from a sensory neuron [102]. At least three theories have been proposed to explain the presence of HSV-1 in the cornea [102]. One theory is operational latency, which was defined by Kennedy *et al.* as “in the animal model 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” [102]. A second theory is that there is a low persistent infection in the cornea, characterized by slow viral replication with small amounts of infectious viral particles released [190]. Detection is not easy with current methods and there are no clinical signs. A third theory is that reactivation from neuronal sites results in the anterograde transport of infectious HSV/HSV-DNA into the cornea [100,102]. Clinically, the possible existence of corneal latency and the understanding of this process are important for the eventual treatment of HK.

Rabbit and human studies suggested that following HK infection, HSV-1 DNA persists in the cornea [101,183]. Penetrating keratoplasty (PKP) or corneal transplant is successful, and is the most common transplant procedure. HK is the cause of approximately 5–10% of PKP and the third leading cause in the USA and UK [191–195]. Cases of newly acquired HK following PKP have been reported [186,196]. Zheng *et al.* investigated this complication in rabbits by transplanting corneas from rabbits with latent HSV-1 to naive rabbits [197]. The corneas of the rabbits were infected with one of two mutant strains of 17 Syn+, either 17Pr (LAT-positive) or 17ΔPst (LAT-negative). After transcorneal epinephrine iontophoresis, retrograde migration of HSV-1 DNA occurred from the transplanted corneas of the rabbits latently infected with 17Pr to the naive uninfected rabbits. HSV-1 DNA was found in 27% (3/11) and 18% (2/11) of the naive rabbit corneal rims and TG, respectively. No migration occurred if the eye was infected with the LAT-negative strain. These results suggested that HSV-1 remains latent in the cornea and upon adrenergic induction is capable of retrograde migration. Latency of the transplanted infected donors was established when there was no detection of viral shedding in tears or the presence of corneal lesions by slit-lamp examination [197]. In their review, Kennedy *et al.* proposed that it was possible that true corneal latency was not established and that at the time of transplant there may have been a very low level infection not detectable, owing to methods of detection being insufficiently sensitive [102].

Recent studies have been performed to determine if human donor transplanted cornea transfers HSV to the recipient and/or if PKP causes an endogenous reactivation of HSV-1 in recipient. Remeijer *et al.* reported a recipient acquiring HSV-1 infection following transplantation of a donor cornea after PKP [198]. The HSV-1 genome was matched between the donor and recipient via PCR. This study demonstrated transmission of HSV-1 from donor to host, suggesting corneal latency; the contamination with HSV-1 was the cause of graft failure. In a retrospective case study, 14 patients were reviewed who presented with new-onset HK after PKP [199]. The authors estimated that ocular HSV occurs six times more frequently in patients who have had PKP. They suggest that the corneal incision (and other corneal trauma) during PKP causes reactivation of latent HSV. Since the innervations

of the corneal epithelium are 300–600 times those of the skin, disruption of the corneal nerve, as occurs in PKP, could provide a strong stimulus for reactivation [199]. The transmission of HSV-1 through PKP suggests the possibility of corneal latency.

Asymptomatic corneas with the presence of HSV-1 DNA and mRNA transcripts have also been evaluated. Robert *et al.* evaluated the outcome of HSV DNA-positive grafts over a 6-month period [186]. Corneal buttons from 38 grafts recipients were analyzed. A total of 8 of 38 (21%) of the corneal buttons were positive for HSV DNA. After transplantation, the healing time of the eight corneas was prolonged from 1.52 to 2.75 days. The authors suggested that either topical steroids (e.g., dexamethasone) given postoperatively or intraoperative trauma caused the reactivation of latent HSV-1 in recipient residual cornea [186]. Finally, a study by Kaye *et al.* [183] found, in five out of ten corneas, the presence of three different HSV-1 DNA sequences in patients who had undergone PKP for nonherpetic reasons. Of those five, three were found by RNA PCR to contain LAT. This finding of three different DNA sequences implies that the entire genome is present and makes latency in the cornea highly likely, owing to the present LAT being the only abundant RNA transcript [183].

Remeijer *et al.* assessed 450 transplant recipients' corneal buttons (83 with HK and 367 without HK), 84 clear bank corneas and 273 corneoscleral rims (remains after trephination of the central cornea) [196]. They reported HSV-1 DNA in 48% (40/83) of patients with HK and 4% (15/367) in patients without HK. In both corneoscleral rims and clear bank corneas, less than 1% was detected. They found that graft failure was increased in patients with HK after PKP, but graft survival was not significantly different. Hill and Clement, in an editorial commentary on the Remeijer *et al.* study [196], noted that the HSV-1 DNA of patients with HK was found to have approximately 100-times more HSV-1 DNA than patients without HK [200]. They offered three possible mechanisms of why a high viral load increases graft failure. First, some humans harbor a latent strain characterized as a high phenotypic reactivator. The second suggested mechanism is that the recipient's corneal rim contains enough HSV-1 DNA to reactivate, migrate to the transplanted cornea and cause an immune response. This eventually leads to a graft failure. The third mechanism is a combination of the first two. This high viral load correlated to preoperative steroid treatment [196]. Hill and Clement noted that in patients with HK, graft failure occurred when intense antiviral and anti-inflammatory treatment was given both pre and post-PKP [200–202]. These results emphasized the need for better chemotherapeutic options. Kennedy *et al.* noted that although HSV-1 DNA was 100-times higher in patients with HK versus patients without HK, there was no evidence of the origin of this HSV-1 DNA and it only suggests evidence of high viral load related to graft failure [102].

What do these data suggest? The presence of HSV-1 DNA, especially in patients with HK, may be suggestive of corneal latency. In patients without HK, the HSV-1 DNA may be the result of neuronal reactivation and the virus 'passing through'. This idea is supported by the finding that approximately 90% of human subjects have asymptomatic shedding of HSV-1 DNA and approximately 90% of human TG contain HSV-1 DNA [14,163].

Reactivation

Reactivation is necessary for completion of the HSV-1 viral cycle; targeting reactivation prevents spreading the virus and disease recurrence, which is caused by reactivation. This constant recurrence of HK causes scar formation on the cornea and the subsequent need for PKP. Stressful stimuli such as UV radiation, fever, stress, hypothermia, hyperthermia and surgical manipulation of the TG can cause reactivation [68]. Not all the neurons in the sensory ganglia reactivate at the same time. Studies reported that reactivation is an isolated

event that is limited to a few neurons in sensory ganglia [203,204]. The frequency at which an individual has reactivation ranges from 0–12 episodes per year and is positively correlated to the amount of TG latently infected [179].

The exact control of reactivation, on the molecular level, has not been elucidated; however, mutations of the HSV genome that can negatively impact both latency and replication have an effect on reactivation. TK is needed for the expression of lytic genes in HSV-1. TK-negative mutant strains cause replication deficits in nondividing cells as a result of reduced expression of both IE and E lytic genes. This same mutation has been found to cause reactivation deficits, suggesting a role for TK [26,205,206]. LAT has also been linked to reactivation. Various studies in mouse and rabbit models have found that mutations in LAT on the 5' end of the promoter and the first 1.5 kb region result in reduced reactivation. However, these recombinant viruses had viral replication equivalent to their parent [26,108,144,145,207].

Many factors and mechanisms have been suggested to be involved in stress-induced reactivation of HSV-1 [66]. This includes the inflammatory cytokine IL-6 and ciliary neurotrophic factor (CNTF) [208–210]. Conversely, nerve growth factor (NGF) is implicated in the inhibition of reactivation [65] as shown by Hill *et al.* that systemic administration of antibodies to NGF induced reactivation of virus in HSV-1 latent rabbits [211].

IL-6 is produced by many different inflammatory cells in response to inflammation. Upon stimulation, IL-6 binds to JAKs that activate transcription factors STATs. The STATs then regulate gene expression [65,208]. Within the LAT and ICP0 promoter, there are IL-6 response elements and possible binding sites for the IL-6-dependent transcription factors, STAT3. HSV-1 constructs with deletion of the IL-6 response element in the LAT promoter reactivate at much lower rates than similar constructs without the deletion [208]. Furthermore, in animal models, systemic administration of an anti-IL-6 antibody significantly reduced the frequency of HSV-1 induced reactivation [208,209]. In the TG, 70–80% of the sensory neurons are dependent on NGF for survival and function [212,213]. Upon stimulation, NGF binds to its receptor trkA to affect gene expression [65]. *In vitro* studies of HSV-1 reactivation in latently infected rat ganglion cells found NGF to have an inhibitory effect. When NGF was removed, HSV-1 antigens and live virus were reactivated [213]. Administration of anti-NGF in rabbit and mouse *in vivo* models resulted in HSV-1 reactivation. Further examining the roles of IL-6 and NGF, Kriesel monitored the expression of trkA and the STATs following TG explantation [65]. He found an increase in the expression of STAT1, 3 and 5b and a decrease in expression in trkA, reinforcing the concept that IL-6 stimulates while NGF inhibits reactivation. Finally, another cytokine, CNTF has been found to have a role in reactivation. This cytokine is unique to nervous tissue and is not actively secreted, but released following peripheral nerve damage to promote repair [210]. In a clinical trial for the treatment of amyotrophic lateral sclerosis, CNTF was discovered serendipitously to stimulate reactivation of HSV. In support of this finding, patients treated with high amounts of CNTF frequently developed herpes labialis in a dose-dependent manner [210].

Spontaneous reactivation

Evidence is increasing, in part due to the high frequency of asymptomatic shedding, that reactivation occurs without known triggers [163,214]. Feldman *et al.* suggested that the latency-reactivation cycle is not simple and that lytic gene expression is present in rare latently infected neurons [215]. In one experiment, thousands of latently infected murine TG neurons were analyzed by a fluorescent probe with HSV-1-specific polyclonal antisera. They found one neuron out of ten TG tested that expressed lytic viral transcripts and

proteins [215]. In addition, focal leukocyte infiltrates surrounded each neuron, suggesting that stimulation was the result of an antigen stimulus. They proposed that this process was caused by 'spontaneous molecular reactivation' [215]. Reactivation could then be due to host cell factors rather than to solely HSV-1 gene expression [215]. Further evidence in support of spontaneous reactivation came from Margolis *et al.* [214]. They examined latently infected TGs with two different viral strains, KOS Δ LAT1.8, a LAT deleted virus and KOS, the parent strain. They found that 6% of KOS latent infected murine TG had lytic detection without induced stimuli. Furthermore, KOS Δ LAT1.8 was found to have the same rate of spontaneous reactivation. This suggests that the region deleted from LAT plays no role in spontaneous molecular reactivation [214].

Induced reactivation

A prostaglandin analog that is used in glaucoma treatment, latanoprost, has been found to induce reactivation in rabbits and humans [216,217]. Kaufman *et al.* found that topically applied latanoprost worsened epithelial herpes and increased the risk of recurrence in rabbits infected with the corticosteroid-sensitive F(MP)E strain [216]. They suggested that latanoprost may be contraindicated in people with a history of HK and that it is similar to corticosteroids in terms of worsening and recurrence. Wand *et al.* presented three case reports of patients with histories of ocular HSV-1 infection treated with latanoprost for their glaucoma [217]. In each patient, HSV-1 reoccurred and was manifesting as dendritic herpes simplex keratitis (HSK). Discontinuation of the latanoprost resulted in resolution of dendritic HSK in all three patients. Furthermore, one of the patients was restarted on latanoprost but with prophylactic antiviral medication at the same time. This combination prevented the recurrence but as soon as the antiviral was stopped, HSK reappeared. The authors suggested that latanoprost may mediate an inflammatory response in the eye. The final common pathway for HSV reactivation may be due to prostaglandins [217]. Further support of this idea was shown when acetylsalicylic acid, a COX-2 inhibitor, was able to prevent heat-stressed reactivation of HSV-1 in mice [218,219].

Research into the role that prostaglandins play in reactivation suggests an interaction between cAMP and LAT. Prostaglandins cause the activation of adenylate cyclase, which in turn increases intracellular cAMP levels. cAMP then binds to protein kinase A, resulting in the phosphorylation of transcription factors [129,130,133]. The CRE is postulated to regulate the gene expression of LATs [129,130,133]. Mouse studies with an HSV-1 recombinant strain 17CRE (contains a mutation in the CRE), found decreased reactivation compared with the parent (17 Syn+) and recombinant (17CREr) strains [133]. A study with rabbits also found decreased reactivation with the CRE mutated strain [129].

Recurrent disease

Reactivation of HSV-1 can occur intermittently (at random). Upon reactivation, the virus is transported axonally to the periphery of the original infecting dermatome and can recur anywhere along the original infected dermatome. The virus then enters the stratified squamous epithelium layer to emerge on the mucosa or skin surface [220]. The virus tends to recur at sites with a high proportion of sensory neuron innervation. These sites include the cornea, lips, fingertips and the oral mucosa. The primary infection is characterized by long-lasting viral replication and shedding. In comparison to the primary infection, the recurrence time is shorter, owing to a quicker acquired immune response; however, owing to the inflammatory response, most of the clinical symptoms of pain, swelling and redness, present during recurrence [221,222]. Irreversible stromal damage may result during the recrudescence herpetic keratitis (RHK) [223].

Factors that contribute to recurrence and severity include the immune status of the host and genetic make-up of both the host and virus. Immunodeficient patients, either acquired or inherited, have higher reactivation and recurrence rates; however, certain immunocompetent hosts have a higher recurrence rate. Pica *et al.* compared peripheral blood mononuclear cells of patients who were HSV-1 seropositive with a history of recurrent herpes labialis to HSV-1 seronegative patients without a history of recurrence [224]. They found much lower levels of IFN- λ in patients with a history of recurrence. Thus, the authors suggested that reduced levels of IFN- λ may have a role in the recurrent response. Host factors that contribute to increased risk of recurrence including gene carriers of the of apoE ϵ 4 [54–56,64]. No specific viral factor or gene has been linked to increased virulence or reactivation; however, certain viral strains have been found in the rabbit model to be low phenotypic reactivators (F, KOS, SC-16, Rodanas, McIntyre, CGA-3) and high phenotypic reactivators (17 Syn+ and McKrae) (Table 4).

The Herpetic Eye Disease Study (HEDS) [225], a 74-center study, determined various risk factors and nonrisk factors for ocular HSV recurrence after following 346 patients from the placebo group of their ACV Prevention Trial [226] for 18 months. All of these patients had experienced an episode of ocular HSV in the previous year. The results are summarized in BOX 3 [225].

Interestingly, a history of epithelial keratitis was not a risk factor for recurrent HSV ocular disease, but a history of stromal keratitis increased the risk. Furthermore, the risk was positively correlated to the number of previous episodes. Patients with two to three previous episodes of stromal keratitis were 1.4-times more likely to have a recurrence than those with only one previous episode and those with four or more were 2.1-times more likely [225].

Although many recurrent diseases are due to reactivation from latency, some studies have documented reinfection with a different (new) strain of HSV-1 at the same site of the primary infection as the cause [121,227,228]. Remeijer *et al.* [223] demonstrated that many RHK are frequently due to a reinfection with a new HSV-1 strain and proposed that PKP may be a risk factor for this superinfection. This is of importance because it has been found that 6.4% of corneal HSV-1 isolates were ACV resistant [229]. It may be due to the widespread use of ACV in the treatment of RHK (those with both latent and superinfections) leading to the selection of HSV-1 resistant strains [230]. A recent study by Duan *et al.* found that isolates of HSV-1 from the cornea of 15 immunocompetent patients with RHK were a mixture of ACV-resistant and -sensitive strains with the same genotype but different TK [230]. The authors concluded that since the same ACV-resistant virus strain was recovered during consecutive RHK episodes, the resistant ACV strain establishes latency and reactivates intermittently. This causes refractory ACV RHK. Furthermore, the authors suggest that ACV appears to contribute to the persistence of these strains. In their conclusion, they suggest that ophthalmologists should be made aware of clinical signs of RHK that are ACV refractory. The susceptibility to ACV of the HSV-1 strain isolated from the cornea should be determined. In case of resistance, switching to a TK-independent antiviral, such as foscarnet and tapering the steroid treatment are recommended [230].

Treatment

Various physical, chemical and antiviral agents have been used to treat HSV epithelial keratitis. In the first half of the 20th century, corneal epithelial debridement and cauterization came into widespread use; now they are no longer used. In 1962, Kaufman *et al.* reported the use of idoxuridine in HSV epithelial keratitis [231]; the success of idoxuridine led to the development of other nucleoside analogs such as vidarabine and trifluorothymidine [232]. ACV was subsequently developed in topical, IV and oral

formulations. Interferons have also been tested for use in therapy and prevention. Ganciclovir (GCV), foscarnet, bromovinyldoxyuridine, cidofovir and other drugs have been recently evaluated for their efficacy in treating dendritic epithelial keratitis [232]. Controversies persist over the optimal antiviral agent and the role of interferon. Complex decision making is involved in selecting appropriate therapy for ocular herpes [232]. **Tables 5–7** summarize human, rabbit and mouse antiherpetic studies [60,164,233–254].

Current regimens

Topical treatment or topical/systemic combinations—In a review, Toma *et al.* suggested that the best evidence-based treatment for HSV epithelial keratitis is topical trifluorothymidine (also known as trifluridine) and topical or oral ACV, with possible adjuvant therapy with topical interferon [66]. For treatment of stromal keratitis, evidence supports the use of topical corticosteroids with a prophylactic antiviral to shorten the duration and long-term suppressive oral ACV therapy to reduce the incidence of recurrent keratitis [202]. Corticosteroids control the immune response of stromal keratitis by inhibiting CD4⁺ cell production of cytokines [255]. This efficacy of corticosteroids was reported in a review by Knickelbein *et al.* to be high and thus outweigh the adverse side effects of possible cataract and glaucoma [256]. In an extensive review, Wilhelmus compared the effects of various therapeutic interventions for dendritic or geographic HSV epithelial keratitis [232]. The review included 99 comparative clinical trials that randomized a total of 5363 participants from 1960 to 2007. This study concluded that the currently available antiviral agents are effective and nearly equivalent. Furthermore, the combination of a nucleoside antiviral with either debridement or interferon appeared to speed up healing. Despite the availability of effective antivirals, a small percentage of patients with recurrent ocular herpes, especially stromal opacities, do not respond to these intense treatments. This finding highlights the need for novel treatment options that target the symptoms as well as asymptomatic shedding of the HSV. Finally, because it is known that HSV-1 tends to recur after ocular surgery in patients with a history of HSV-1 keratitis, prophylactic treatment is recommended for these patients before undergoing corneal surgery. In a retrospective study, topical and systemic antiviral therapies are suggested to be used postoperatively for at least one year after penetrating keratoplasty [257].

Physiochemical therapy—Physiochemical therapies vary widely. The extent of physiochemical de-epithelialization varies from wiping loosened, infected epithelial cells by minimal debridement to thermomechanical or cryomechanical curettage and chemical cauterization [232]. Wilhelmus concluded that minimal wiping debridement without antiviral cotreatment was often followed by recrudescence epithelial keratitis, while more aggressive physiochemical methods apparently removed all infected epithelial cells [232]. However, debridement did not reduce the risk of subsequent HSV stromal keratitis. Drawbacks associated with these methods include the time and effort needed for the procedure, the risk of damaging Bowman's layer, the enhancement of corneal inflammation or scarring and the occurrence of recrudescence epithelial keratitis if additional antiviral therapy was not provided [232].

Systemic treatment—Wilhelmus found that most clinical trials of the antiviral treatment of HSV epithelial keratitis compared different antiviral agents but no single treatment could be used as a standard reference [232]. He reported that idoxuridine was superior to placebo; however, variable formulations and dosages were likely to have contributed to heterogeneity among the placebo-controlled trials. Idoxuridine was removed from the market in the early 1990s owing to low demand as other antivirals were introduced. Wilhelmus also concluded that compared with idoxuridine, the topical application of vidarabine, trifluridine, ACV or GCV generally resulted in a significantly greater proportion of participants healing within

one week of treatment [232]. Among these four antiviral agents, no treatment emerged as significantly better for the therapy of dendritic epithelial keratitis. Sufficient data were not available to adequately assess topical bromovinyldeoxyuridine or oral ACV although these agents appeared equivalent to topical trifluridine or topical ACV. The addition of an adjunctive agent, such as topical vidarabine or oral ACV, to topical antiviral therapy did not significantly improve epithelial healing. Short-term therapeutic use of a topical or oral antiviral agent did not reduce the risk of recurrent epithelial or stromal keratitis following treatment of dendritic epithelial keratitis [232]. **Box 3 & Tables 4 & 5** summarize past antiherpetic studies in human, rabbits and mice.

Antivirals such as ACV, valacyclovir and famciclovir have been reported to reduce HSV-2 DNA shedding in genital herpes [258]. However, none of the studies or other antiviral studies demonstrated complete suppression of HSV-1, HSV-2 or VZV DNA shedding or lesion prevention [259]. Loutsch *et al.* evaluated the efficacy of famciclovir in the treatment of HSV keratitis in rabbits [252]. They found that famciclovir reduced the corneal lesions and the amount of HSV-1 latent in the TG and proposed famciclovir as a possible prophylactic treatment in patients with recurrent HSV keratitis [249]. In addition, COX inhibitors, such as aspirin, have been reported to reduce the clinical signs of herpes infections of the skin, eyes and genitals [259]. Other studies have demonstrated suppression of HSV-1 reactivation by COX inhibitors in mice latently infected with HSV-1 [219]. Yin *et al.* using a corneal mouse model reported that the COX inhibitor lornoxicam prevented recurrence of HSK and offered protection by preventing corneal opacity and downregulating NK- κ B [260]. This suggests the possibility of using COX inhibitors prophylactically to prevent recurrence and shedding. However, Kumar *et al.* reported that neither valacyclovir alone nor valacyclovir combined with aspirin reduced the asymptomatic shedding of HSV-1 DNA in the tears and saliva of healthy individuals [164]. They also concluded that HSV-1 DNA shedding from tears and saliva was not related to the antibody titer against HSV-1, race, sex or age. Based on this study, the prophylactic antiviral appears to not be useful in HSV-1 infections in preventing transmission of the virus. In another study by Kumar *et al.*, the effect of high versus low oral doses of valacyclovir on HSV-1 DNA shedding into tears of latently infected rabbits was investigated (**Table 8**) [248].

They found that doses of 70 and 140 mg/kg of valacyclovir significantly reduced the HSV-1 DNA copy numbers. A dose of 7 mg/kg daily (roughly equivalent to a 500-mg dose in humans) did not suppress HSV-1 DNA shedding. In addition, no visible side effects, toxicity or mortality were observed in the rabbits treated with valacyclovir doses as high as 140 mg/kg. Based on these findings, Kumar *et al.* [248] proposed that a higher dose of valacyclovir might be necessary to reduce asymptomatic shedding and virus transmission. These data are significant owing to the evidence that greater than 90% of the human population has HSV-1 DNA in their TG and asymptotically shed DNA into their tears and saliva [163]. This shedding is a likely source of disease transmission and, thus, suppression of asymptomatic shedding is one way that transmission of this virus might be blocked. In conclusion, further research is needed to find a prophylactic agent, either an antiviral, COX inhibitor or a combination.

A valacyclovir dose of 2 g four times daily (8 g/d) has been reported to cause mild adverse side effects in immunocompetent subjects [261,262]. A recent review indicates possible neuropsychiatric adverse effects in elderly patients and those with acute or chronic renal failure [263]. A potentially fatal thrombotic microangiopathy-like syndrome has been reported in some immunocompromised patients receiving high-dose prophylactic valacyclovir therapy (8 g/d) for cytomegalovirus disease for prolonged periods [261]. It is recognized that not everyone can tolerate high-dose valacyclovir treatment.

To date only two antiviral drugs are FDA approved for topical treatment of HSV-1 epithelial keratitis, trifluridine and GCV [264,265]. Trifluridine, in a 1% topical solution, has historically been the drug of choice and the only topical form approved until 2009. Both GCV and ACV/valacyclovir have a similar mechanism of action and they both require phosphorylation by viral TK. Trifluridine does not require activation by viral TK, hence it is effective against ACV/valacyclovir-resistant strains [266]. Although trifluridine is superior in terms of its effectiveness against ACV-resistant strains, it has adverse effects, such as an increase in intraocular pressure, corneal edema and a burning sensation upon application. However, GCV is reported to have fewer of these particular side effects [267]. Hobden *et al.* tested *in vitro* the synergism of trifluridine and GCV in a 10:1 ratio, respectively, against three laboratory and nine clinical strains of HSV-1 [266]. They found that for all strains, the combination was effective. Also, the combination was synergistic against an ACV-susceptible strain. Furthermore, the concentrations used for both drugs were much less toxic than if each had been used alone. Although synergism against HSV between trifluridine and other compounds such as INF- α [268], docosanol [269] and docetaxel [270] have been reported, none these agents are used topically. More experiments are needed to study these two drugs, especially *in vivo* models [266].

New therapies

Interferon—Wilhelmus found that interferon monotherapy had a slightly beneficial effect on dendritic epithelial keratitis but was not better than other antiviral agents [232]. Topical interferon was useful with debridement. The likelihood of treatment success appeared to be increased when a combination of a topical interferon with a topical nucleoside analog antiviral agent such as trifluridine or ACV was administered [232].

Amniotic membrane transplantation—Amniotic membrane transplantation (AMT) has been studied as a possible treatment for stromal keratitis. One study evaluated the treatment outcome of 15 patients with necrotizing stromal keratitis following AMT with antivirals and corticosteroids [271]. They found that 14/15 (93%) displayed improved visual acuity over a 7–13-month period, and paracentral and central ulcers healed after 2 weeks. In a review of this study, Knickelbein *et al.* concluded that since all patients were treated with corticosteroids as well, it was not clear whether AMT or the steroids were responsible for the therapeutic effect [256]. More studies with AMT are required, especially in patients with stromal keratitis refractive to antiviral and corticosteroid treatment.

ApoE, peptides, enzyme inhibitors, Zyban/Wellbutrin & combinations—Other possible new therapies include peptides derived from receptor-binding regions of human apoE. Since carriers of apoE ϵ 4 are more susceptible to inflammatory-related diseases and viral infections, it was thought that apoE peptide could possibly be used to treat HSK. Bhattacharjee *et al.* compared topical treatment of HSK with 1% apoE mimetic dimer peptide (dp), 1% trifluridine and phosphate-buffered saline on mice corneally infected with HSV-1 strain KOSGFP [61]. Based on slit-lamp, immunologic and virological evaluation, apoEdp appeared to be anti-inflammatory and anti-HSV-1 by blocking development and progression of HSK. Similar shedding was also found to be decreased. In fact, when compared with 1% topical trifluridine, the results were similar [61]. In a recent study, Bhattacharjee *et al.* found that apoEdp inhibited VEGF-induced angiogenesis in rabbits corneally implanted with VEGF pellets and prevented tumor growth in mice [62]. *In vitro* they found that the peptide inhibited migration, invasion, endothelial cell proliferation and capillary tube formation. These results are the first evidence to our knowledge of the possible anti-angiogenic function of apoEdp. ApoEdp could be used one day not only in the treatment of HSK, but also as an anti-tumor agent.

The antiviral effects of peptides against HSV-1 have been shown. Peptides have been found to be a major component of the vertebrate innate host defense system [272]. In mammals, the two main families of peptides are cathelicidins [273] and defensins [274]. These peptides have been shown to inhibit entry and infection of the HSV-1 virus [275–278]. A synthetic cationic θ -defensin, retrocyclin (RC-2), was found to have activity in a mouse model study [253]. Applying RC-2 to the cornea in 2% methylcellulose before HSV-1 infection or incubating the peptide with HSV-1, greatly reduced the severity of the ocular infection. However, after viral infection, treatment with RC-2 did not reduce the severity. The authors concluded that RC-2 may help prevent entry of the virus and not replication, and it may have a possible use as a prophylactic agent (**Table 7**)[253].

Monoamine oxidase inhibitors (MAOIs) have recently been investigated for use in the treatment of HSV-1 infections. A cellular transcription coactivator, host cell factor-1, is needed to recruit lysine-specific demethylase (LSD)-1 for the expression of HSV IE genes [151,279–281]. LSD-1 is brought to the IE gene promoter and needed for HSV-1 gene expression. MAOI have been found to deplete LSD-1. This depletion by the MAOI in an *in vitro* assay prevents viral transcription of the IE genes, thus preventing the virus from reactivating from latency [282]. The use of MAOI is a new possible pharmaceutical control to prevent reactivation and asymptomatic shedding of HSV [151,282].

The development of a vaccine has been investigated for use against HSV-1 to prevent recurrent herpetic ocular disease and asymptomatic shedding [36,283]. A human study carried out by Pivetti-Pezzi *et al.* [283] found that vaccination with a heat-inactivated viral strain of HSV-1 reduced the duration and number of recurrences in patients with a history of HSV keratitis compared with a control group. However, this study only evaluated the vaccine in 10 patients and the reduction in duration and recurrence was not statistically significant compared with the control group. Critics of the study suggested that a future study is needed with a larger cohort [256]. Bettahi *et al.* vaccinated mice with self-adjuvanting glycoprotein D lipopeptides [284]. The vaccine containing T-cell epitopes driving a CD4⁺ T-cell response was found to protect the mice from HSV keratitis when vaccinated before primary infections. The tear viral titer was reduced in vaccinated mice, but there was an increase in HSV-1 CD4⁺ T-cell proliferation and cytokines [284]. Critics of this study are concerned that the increase in CD4⁺ T cells may exacerbate a stromal keratitis infection in individuals already infected [256]. Although both studies have their drawbacks, the research is still promising for the potential of an antiherpetic vaccine.

Recurrence

Risk of recurrence is more common after ocular surgery; therefore, prophylactic treatment for patients with a history of herpes simplex keratitis undergoing corneal surgery is recommended. Topical and systemic antiviral therapies are suggested to be used postoperatively for at least one year after penetrating keratoplasty [257].

Conclusion

Most humans harbor HSV-1 DNA in ganglionic neurons and non-neuronal sites such as the cornea. Furthermore, most humans exhibit asymptomatic shedding based on studies from tears, saliva and vaginal secretions. This asymptomatic shedding is one means to account for the high percentage of humans infected. To prevent asymptomatic shedding and symptomatic outbreaks, studies have found that the best chemotherapy options are high doses of antiherpetics such as valacyclovir and/or combination therapies. Combination therapies reported to be the most effective in animal models include a COX inhibitor such as aspirin and corticosteroids as well as the standard antiherpetics with different mechanisms.

Although molecular (operational) latency differs from practical latency, both definitions state that no infectious HSV-1 is present; however, LAT continues to be expressed. In certain individuals, reactivation from latency occurs more frequently. Certain genetic characteristics, such as the apoE e4 allele, tend to have a higher rate of infection, reactivation and recurrence. Furthermore, the phenotype of HSV-1 strain that causes the infection has been related to reactivation frequency. Certain HSV-1 strains are high phenotypic reactivators while others are low. Higher phenotypic reactivators tend to have more spontaneous asymptomatic shedding in tears and saliva than low phenotypic reactivators. Although the current treatment regimens of ocular HSV generally are effective, more research and treatment options are needed to help prevent asymptomatic shedding and serious recurrences. New therapies on the horizon include apoE peptides, monamine oxidase inhibitors and vaccines against HSV-1.

Future perspective

In the next 5–10 years, chemotherapy of ocular herpes will improve, even though present treatment is very good. Therapy will certainly utilize combinations of drugs that will consider the genetics of the host and the virus. Most likely for the refractive and/or resistant cases of ocular herpes, at least four classes of drugs will be employed. Of course, the primary drug will be a classic antiherpetic such as acycloguanosine. Another drug class could be a mimetic peptide such as that derived from ApoE. Most likely an anti-inflammatory and antineovascularization drugs will also be utilized. The outcome of these combinations will be more successful than the current therapy.

Bibliography

Papers of special note have been highlighted as:

■ of interest

■ of considerable interest

1. Xu F, Sternberg MR, Kottiri BJ, et al. Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *JAMA*. 2006; 296(8):964–973. [PubMed: 16926356]
2. Roizman, B.; Knipe, DM.; Whitley, RJ. Herpes simplex viruses.. In: Knipe, DM.; Howley, PM., editors. *Fields Virology*. 5th Edition. Lippincott Williams & Wilkins; Baltimore, MD, USA: 2007. p. 2503-2602.
3. Kaye S, Choudhary A. Herpes simplex keratitis. *Prog. Retin. Eye Res.* 2006; 25:355–380. [PubMed: 16807055]
4. Nesburn, AB. Vision Research – A National Plan, 1994–1998. US Department of Health and Human Services, Public Health Service, National Institutes of Health and the National Eye Institute; Bethesda, MD, USA: 1997. Report of the corneal disease panel; p. 138-146.
5. Ball MJ, Mathews R, Steiner I, et al. Latent HSV 1 virus in trigeminal ganglia: the optimal site for linking prevention of Alzheimer's disease to vaccination. *Neurobiol. Aging*. 2001; 22(5):705–709. [PubMed: 11705627]
6. Bustos DE, Atherton SS. Detection of herpes simplex virus type 1 in human ciliary ganglia. *Invest. Ophthalmol. Vis. Sci.* 2002; 43(7):2244–2249. [PubMed: 12091423]
7. Cohrs RJ, Randall J, Smith J, et al. Analysis of individual human trigeminal ganglia for latent herpes simplex virus type 1 and varicella-zoster virus nucleic acids using real-time PCR. *J. Virol.* 2000; 74(24):11464–11471. [PubMed: 11090142]
8. Efsthathiou S, Minson AC, Field HJ, Anderson JR, Wildy P. Detection of herpes simplex virus-specific DNA sequences in latently infected mice and in humans. *J. Virol.* 1986; 57(2):446–455. [PubMed: 3003377]

9. Liedtke W, Opalka B, Zimmermann CW, Lignitz E. Age distribution of latent herpes simplex virus 1 and varicella-zoster virus genome in human nervous tissue. *J. Neurol. Sci.* 1993; 116(1):6–11. [PubMed: 8389816]
10. Mahalingam R, Wellish MC, Dueland AN, Cohrs RJ, Gilden DH. Localization of herpes simplex virus and varicella zoster virus DNA in human ganglia. *Ann. Neurol.* 1992; 31(4):444–448. [PubMed: 1316733]
11. Motani H, Sakurada K, Ikegaya H, et al. Detection of herpes simplex virus type 1 DNA in bilateral human trigeminal ganglia and optic nerves by polymerase chain reaction. *J. Med. Virol.* 2006; 78(12):1584–1587. [PubMed: 17063515]
12. Pevsner SR, Williams RK, McChesney D, Mont EK, Smialek JE, Straus SE. Quantitation of latent varicella-zoster virus and herpes simplex virus genomes in human trigeminal ganglia. *J. Virol.* 1999; 73(12):10514–10518. [PubMed: 10559370]
13. Verjans GM, Hintzen RQ, van Dun JM, et al. Selective retention of herpes simplex virus-specific T cells in latently infected human trigeminal ganglia. *Proc. Natl Acad. Sci. USA.* 2007; 104(9): 3496–3501. [PubMed: 17360672]
14. Hill JM, Ball MJ, Neumann DM, et al. The high prevalence of HSV-1 DNA in human trigeminal ganglia is not a function of age or gender. *J. Virol.* 2008; 82(16):8230–8234. [PubMed: 18550674] [Showed that 90% of human trigeminal ganglia contains herpes simplex virus (HSV) type 1 DNA.]
15. Liesegang TJ. Classification of herpes simplex virus keratitis and anterior uveitis. *Cornea.* 1999; 18(2):127–143. [PubMed: 10090358]
16. Holland EJ, Schwartz GS. Classification of herpes simplex virus keratitis. *Cornea.* 1999; 18(2): 144–154. [PubMed: 10090359]
17. Lepisto AJ, Frank GM, Hendricks RL. How herpes simplex virus type 1 rescinds corneal privilege. *Chem. Immunol. Allergy.* 2007; 92:203–212. [PubMed: 17264496]
18. Liesegang TJ. Epidemiology of ocular herpes simplex. Natural history in Rochester, Minn, 1950 through 1982. *Arch. Ophthalmol.* 1989; 107:1160–1165. [PubMed: 2757546]
19. Norn MS. Dendritic (herpetic) keratitis. I. Incidence – seasonal variations – recurrence rate – visual impairment – therapy. *Acta Ophthalmol.* 1970; 48:91–107. [PubMed: 5467720]
20. Miserocchi E, Waheed NK, Dios E, et al. Visual outcome in herpes simplex virus and varicella zoster virus uveitis: a clinical evaluation and comparison. *Ophthalmology.* 2002; 109(8):1532–1537. [PubMed: 12153807]
21. Dix RD, McKendall RR, Baringer JR. Comparative neurovirulence of herpes simplex virus type 1 strains after peripheral or intracerebral inoculation of BALB/c mice. *Infect. Immun.* 1983; 40(1): 103–112. [PubMed: 6299955]
22. Levine, M.; Fink, DJ.; Ramakrishnan, R.; Desai, P.; Goins, WF.; Glorioso, JC. Neurovirulence of herpes simplex virus type 1 accessory gene mutants.. In: Becker, Y.; Darai, G., editors. *Pathogenicity of Human Herpesviruses Due to Specific Pathogenicity Genes.* Springer-Verlag; Berlin, Germany: 1994. p. 222-237.
23. Wander AH, Bubel HC, McDowell SG. The pathogenesis of herpetic ocular disease in the guinea pig. *Arch. Virol.* 1987; 95(3–4):197–209. [PubMed: 3038053]
24. Wander AH, Centifanto YM, Kaufman HE. Strain specificity of clinical isolates of herpes simplex virus. *Arch. Ophthalmol.* 1980; 98(8):1458–1461. [PubMed: 6251792]
25. Sawtell NM. Comprehensive quantification of herpes simplex virus latency at the single-cell level. *J. Virol.* 1997; 71(7):5423–5431. [PubMed: 9188614]
26. Sawtell NM, Poon DK, Tansky CS, Thompson RL. The latent herpes simplex virus type 1 genome copy number in individual neurons is virus strain specific and correlates with reactivation. *J. Virol.* 1998; 72(7):5343–5350. [PubMed: 9620987]
27. Ecob-Prince MS, Rixon FJ, Preston CM, Hassan K, Kennedy PGE. Reactivation *in vivo* and *in vitro* of herpes simplex virus from mouse dorsal root ganglia which contain different levels of latency-associated transcripts. *J. Gen. Virol.* 1993; 74(Pt 6):995–1002. [PubMed: 8389814]
28. Maggioncalda J, Mehta A, Su YH, Fraser NW, Block TM. Correlation between herpes simplex virus type 1 rate of reactivation from latent infection and the number of infected neurons in trigeminal ganglia. *Virology.* 1996; 225(1):72–81. [PubMed: 8918535]

29. Centifanto-Fitzgerald YM, Yamaguchi T, Kaufman HE, Tognon M, Roizman B. Ocular disease pattern induced by herpes simplex virus is genetically determined by a specific region of viral DNA. *J. Exp. Med.* 1982; 155(2):475–489. [PubMed: 6276491]
30. Brandt CR. Mixed ocular infections identify strains of herpes simplex virus for use in genetic studies. *J. Virol. Methods.* 1991; 35(2):127–135. [PubMed: 1667784]
31. Brandt CR, Grau DR. Mixed infection with herpes simplex virus type 1 generates recombinants with increased ocular and neurovirulence. *Invest. Ophthalmol. Vis. Sci.* 1990; 31(11):2214–2223. [PubMed: 2173682]
32. Brandt CR, Kolb AW, Shah DD, et al. Multiple determinants contribute to the virulence of HSV ocular and CNS infection and identification of serine 34 of the US1 gene as an ocular disease determinant. *Invest. Ophthalmol. Vis. Sci.* 2003; 44(6):2657–2668. [PubMed: 12766070]
33. Caspary L, Schindling B, Dundarov S, Falke D. Infections of susceptible and resistant mouse strains with herpes simplex virus type 1 and 2. *Arch. Virol.* 1980; 65(3–4):219–227. [PubMed: 6251783]
34. Stulting RD, Kindle JC, Nahmias AJ. Patterns of herpes simplex keratitis in inbred mice. *Invest. Ophthalmol. Vis. Sci.* 1985; 26(10):1360–1367. [PubMed: 4044164]
35. Whitley RJ. Herpes simplex virus infection. *Semin. Pediatr. Infect. Dis.* 2002; 13(1):6–11. [PubMed: 12118847]
36. Koelle DM, Corey L. Recent progress in herpes simplex virus immunobiology and vaccine research. *Clin. Microbiol. Rev.* 2003; 16(1):96–113. [PubMed: 12525427]
37. Fujihara T, Hayashi K. Lactoferrin inhibits herpes simplex virus type-1 (HSV-1) infection to mouse cornea. *Arch. Virol.* 1995; 140(8):1469–1472. [PubMed: 7661698]
38. Lopez C. Genetics of natural resistance to herpesvirus infections in mice. *Nature.* 1975; 258(5531):152–153. [PubMed: 171586]
39. Wu L, Morahan PS. Macrophages and other nonspecific defenses: role in modulating resistance against herpes simplex virus. *Curr. Top. Microbiol. Immunol.* 1992; 179:89–110. [PubMed: 1499352]
40. Lundberg P, Welander P, Openshaw H, et al. A locus on mouse chromosome 6 that determines resistance to herpes simplex virus also influences reactivation, while an unlinked locus augments resistance of female mice. *J. Virol.* 2003; 77(21):11661–11673. [PubMed: 14557652]
41. Jouanguy E, Zhang SY, Chapgier A, et al. Human primary immunodeficiencies of type I interferons. *Biochimie.* 2007; 89(6–7):878–883. [PubMed: 17561326]
42. Menachery VD, Pasieka TJ, Leib DA. Interferon regulatory factor 3-dependent pathways are critical for control of herpes simplex virus type 1 central nervous system infection. *J. Virol.* 2010; 84(19):9685–9694. [PubMed: 20660188]
43. Hoshino Y, Pesnicak L, Cohen JI, Straus SE. Rates of reactivation of latent herpes simplex virus from mouse trigeminal ganglia *ex vivo* correlate directly with viral load and inversely with number of infiltrating CD8+ T cells. *J. Virol.* 2007; 81(15):8157–8164. [PubMed: 17522198]
44. Lopez C. Resistance to HSV-1 in the mouse is governed by two major, independently segregating, non-*H-2* loci. *Immunogenetics.* 1980; 11(1):87–92. [PubMed: 6254873]
45. Brandt CR. The role of viral and host genes in corneal infection with herpes simplex virus type 1. *Exp. Eye Res.* 2005; 80(5):607–621. [PubMed: 15862167]
46. Hobbs MR, Jones BB, Otterud BE, Leppert M, Kriesel JD. Identification of a herpes simplex labialis susceptibility region on human chromosome 21. *J. Infect. Dis.* 2008; 197(3):340–346. [PubMed: 18199027]
47. Jabbar AA, al-Samarai AM, al-Amar NS. HLA antigens associated with susceptibility to herpes simplex virus infection. *Dis. Markers.* 1991; 9(5):281–287. [PubMed: 1797451]
48. Elner VM, Dutt S, Pavilack MA, Sugar A, Foster CS, Elner SG. Intercellular adhesion molecule-1 (ICAM-1) and HLA-DR antigens in herpes keratitis. *Ophthalmology.* 1992; 99(9):1400–1407. [PubMed: 1357614]
49. Lekstrom-Himes JA, Hohman P, Warren T, et al. Association of major histocompatibility complex determinants with the development of symptomatic and asymptomatic genital herpes simplex virus type 2 infections. *J. Infect. Dis.* 1999; 179(5):1077–1085. [PubMed: 10191207]

50. Kriesel JD, Jones BB, Matsunami N, et al. C21orf91 genotypes correlate with herpes simplex labialis (cold sore) frequency: description of a cold sore susceptibility gene. *J. Infect. Dis.* 2011 In Press. [Identifies a specific human gene that is correlated with herpetic cold sores.]
51. Burgos JS, Ramirez C, Sastre I, Bullido MJ, Valdivieso F. Involvement of apolipoprotein E in the hematogenous route of herpes simplex virus type 1 to the central nervous system. *J. Virol.* 2002; 76(23):12394–12398. [PubMed: 12414984]
52. Burgos JS, Ramirez C, Sastre I, Bullido MJ, Valdivieso F. ApoE4 is more efficient than E3 in brain access by herpes simplex virus type 1. *NeuroReport.* 2003; 14(14):1825–1827. [PubMed: 14534428]
53. Burgos JS, Ramirez C, Sastre I, Valdivieso F. Effect of apolipoprotein E on the cerebral load of latent herpes simplex virus type 1 DNA. *J. Virol.* 2006; 80(11):5383–5387. [PubMed: 16699018]
54. Lin WR, Shang D, Wilcock GK, Itzhaki RF. Alzheimer's disease, herpes simplex virus type 1, cold sores and apolipoprotein E4. *Biochem. Soc. Trans.* 1995; 23(4):594S. [PubMed: 8654779]
55. Hill JM, Bhattacharjee PS, Neumann DM. Apolipoprotein E alleles can contribute to the pathogenesis of numerous clinical conditions including HSV-1 corneal disease. *Exp. Eye Res.* 2007; 84(5):801–811. [PubMed: 17007837]
56. Bhattacharjee PS, Neumann DM, Foster TP, et al. Effect of human apolipoprotein E genotype on the pathogenesis of experimental ocular HSV-1. *Exp. Eye Res.* 2008; 87(2):122–130. [PubMed: 18572164]
57. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* 1988; 240(4852):622–630. [PubMed: 3283935]
58. Mahley RW, Rall SC Jr. Apolipoprotein E: Far more than a lipid transport protein. *Annu. Rev. Genomics Hum. Genet.* 2000; 1:507–537. [PubMed: 11701639]
59. Roses AD. Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Annu. Rev. Med.* 1996; 47:387–400. [PubMed: 8712790]
60. Bhattacharjee PS, Neumann DM, Foster TP, et al. Effective treatment of ocular HSV with a human apolipoprotein E mimetic peptide in a mouse eye model. *Invest. Ophthalmol. Vis. Sci.* 2008; 49(10):4263–4268. [PubMed: 18515564]
61. Bhattacharjee PS, Neumann DM, Hill JM. A human apolipoprotein E mimetic peptide effectively inhibits HSV-1 TK-positive and TK-negative acute epithelial keratitis in rabbits. *Curr. Eye Res.* 2009; 34(2):99–102. [PubMed: 19219680]
62. Bhattacharjee PS, Huq TS, Mandal TK, et al. A novel peptide derived from human apolipoprotein E is an inhibitor of tumor growth and ocular angiogenesis. *PLoS One.* 2010; 6(1):e15905. [PubMed: 21253017]
63. Bhattacharjee PS, Neumann DM, Stark D, Thompson HW, Hill JM. Apolipoprotein E modulates establishment of HSV-1 latency and survival in a mouse ocular model. *Curr. Eye Res.* 2006; 31(9):703–708. [PubMed: 16966142]
64. Lin WR, Tullo AB, Itzhaki RF. Apolipoprotein E and herpes virus diseases: herpes simplex keratitis. *Eur. J. Hum. Genet.* 1999; 7(3):401–403. [PubMed: 10234519]
65. Kriesel JD. The roles of inflammation, STAT transcription factors, and nerve growth factor in viral reactivation and herpes keratitis. *DNA Cell Biol.* 2002; 21(5–6):475–481. [PubMed: 12167251]
66. Toma HS, Murina AT, Areaux RG Jr, et al. Ocular HSV-1 latency, reactivation and recurrent disease. *Semin. Ophthalmol.* 2008; 23(4):249–273. [PubMed: 18584563]
67. Itzhaki RF, Wozniak MA. Herpes simplex virus type 1, apolipoprotein E, and cholesterol: a dangerous liaison in Alzheimer's disease and other disorders. *Prog. Lipid Res.* 2006; 45(1):73–90. [PubMed: 16406033]
68. Spruance SL, Kriesel JD, Evans TG, McKeough MB. Susceptibility to herpes labialis following multiple experimental exposures to ultraviolet radiation. *Antiviral Res.* 1995; 28(1):57–67. [PubMed: 8585760]
69. Lam Q, Smibert CA, Koop KE, et al. Herpes simplex virus VP16 rescues viral mRNA from destruction by the virion host shutoff function. *EMBO J.* 1996; 15(10):2575–2581. [PubMed: 8665865]
70. Alwine JC, Steinhart WL, Hill CW. Transcription of herpes simplex type 1 DNA in nuclei isolated from infected HEP-2 and KB cells. *Virology.* 1974; 60(1):302–307. [PubMed: 4366499]

71. Costanzo F, Campadelli-Fiume G, Foa-Tomasi L, Cassai E. Evidence that herpes simplex virus DNA is transcribed by cellular RNA polymerase B. *J. Virol.* 1977; 21(3):996–1001. [PubMed: 191658]
72. Honess RW, Roizman B. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. *J. Virol.* 1974; 14(1):8–19. [PubMed: 4365321]
73. DeLuca NA, Schaffer PA. Activation of immediate-early, early, and late promoters by temperature-sensitive and wild-type forms of herpes simplex virus type 1 protein ICP4. *Mol. Cell Biol.* 1985; 5(8):1997–2208. [PubMed: 3018543]
74. Gu B, DeLuca N. Requirements for activation of the herpes simplex virus glycoprotein C promoter *in vitro* by the viral regulatory protein ICP4. *J. Virol.* 1994; 68(12):7953–7965. [PubMed: 7966586]
75. Smith CA, Bates P, Rivera-Gonzalez R, Gu B, DeLuca NA. ICP4, the major transcriptional regulatory protein of herpes simplex virus type 1, forms a tripartite complex with TATA-binding protein and TFIIB. *J. Virol.* 1993; 67(8):4676–4687. [PubMed: 8392607]
76. Gelman IH, Silverstein SJ. Herpes simplex virus immediate-early promoters are responsive to virus and cell *trans*-acting factors. *J. Virol.* 1987; 61(7):2286–2296. [PubMed: 3035226]
77. Muller MT. Binding of the herpes simplex virus immediate-early gene product ICP4 to its own transcription start site. *J. Virol.* 1987; 61(3):858–865. [PubMed: 3027412]
78. Boutell C, Everett RD. The herpes simplex virus type 1 (HSV-1) regulatory protein ICP0 interacts with and ubiquitinates p53. *J. Biol. Chem.* 2003; 278(38):36596–36602. [PubMed: 12855695]
79. Cai W, Astor TL, Liptak LM, Cho C, Coen DM, Schaffer PA. The herpes simplex virus type 1 regulatory protein ICP0 enhances virus replication during acute infection and reactivation from latency. *J. Virol.* 1993; 67(12):7501–7512. [PubMed: 8230470]
80. Jerome KR, Fox R, Chen Z, Sarkar P, Corey L. Inhibition of apoptosis by primary isolates of herpes simplex virus. *Arch. Virol.* 2001; 146(11):2219–2225. [PubMed: 11765923]
81. Everett RD, Orr A, Elliott M. High level expression and purification of herpes simplex virus type 1 immediate early polypeptide Vmw110. *Nucleic Acids Res.* 1991; 19(22):6155–6161. [PubMed: 1659686]
82. Gu H, Liang Y, Mandel G, Roizman B. Components of the REST/CoREST/histone deacetylase repressor complex are disrupted, modified, and translocated in HSV-1-infected cells. *Proc. Natl Acad. Sci. USA.* 2005; 102(21):7571–7576. [PubMed: 15897453]
83. Poon APW, Gu H, Roizman B. ICP0 and the US3 protein kinase of herpes simplex virus 1 independently block histone deacetylation to enable gene expression. *Proc. Natl Acad. Sci. USA.* 2006; 103(26):9993–9998. [PubMed: 16785443]
84. Divito S, Cherpes TL, Hendricks RL. A triple entente: virus, neurons, and CD8⁺ T cells maintain HSV-1 latency. *Immunol. Res.* 2006; 36(1–3):119–126. [PubMed: 17337772]
85. Hill A, Jugovic P, York I, et al. Herpes simplex virus turns off the TAP to evade host immunity. *Nature.* 1995; 375(6530):411–415. [PubMed: 7760935]
86. Goldsmith K, Chen W, Johnson DC, Hendricks RL. Infected cell protein (ICP)47 enhances herpes simplex virus neurovirulence by blocking the CD8⁺ T cell response. *J. Exp. Med.* 1998; 187(3):341–348. [PubMed: 9449714]
87. Bloom DC, Giordani NV, Kwiatkowski DL. Epigenetic regulation of latent HSV-1 gene expression. *Biochim. Biophys. Acta.* 2010; 1799(3–4):246–256. [PubMed: 20045093]
88. Guess S, Stone DU. Evidence-based treatment of herpes simplex virus keratitis: a systematic review. *Ocul. Surf.* 2007; 5(3):240–250. [PubMed: 17660897]
89. Knotts FB, Cook ML, Stevens JG. Pathogenesis of herpetic encephalitis in mice after ophthalmic inoculation. *J. Infect. Dis.* 1974; 130(1):16–27. [PubMed: 4366287]
90. Baringer JR. Herpes simplex virus infection of nervous tissue in animals and man. *Prog. Med. Virol.* 1975; 20:1–26. [PubMed: 172970]
91. Baringer JR, Swoveland P. Recovery of herpes-simplex virus from human trigeminal ganglions. *N. Engl. J. Med.* 1973; 288(13):648–650. [PubMed: 4347057]

92. Kramer MF, Chen SH, Knipe DM, Coen DM. Accumulation of viral transcripts and DNA during establishment of latency by herpes simplex virus. *J. Virol.* 1998; 72(2):1177–1185. [PubMed: 9445016]
93. Cohrs, RJ.; Koelle, DM.; Schuette, MC., et al. Asymptomatic alphaherpesvirus reactivation.. In: Gluckman, TR., editor. *Herpesviridae: Viral Structure, Life Cycle and Infections*. Nova Science Publishers Inc.; Hauppauge, NY, USA: 2009. p. 133-168.
94. Perng GC, Jones C. Towards an understanding of the herpes simplex virus type 1 latency-reactivation cycle. *Interdiscip. Perspect. Infect. Dis.* 2010;262415. [PubMed: 20169002]
95. Izumi KM, McKelvey AM, Devi-Rao GB, Wagner EK, Stevens JG. Molecular and biological characterization of a type 1 herpes simplex virus (HSV-1) specifically deleted for expression of the latency-associated transcript (LAT). *Microb. Pathog.* 1989; 7(2):121–134. [PubMed: 2556619]
96. Javier RT, Stevens JG, Disette VB, Wagner EK. A herpes simplex virus transcript abundant in latently infected neurons is dispensable for establishment of the latent state. *Virology.* 1988; 166(1):254–257. [PubMed: 2842950]
97. Thompson RL, Sawtell NM. The herpes simplex virus type 1 latency-associated transcript gene regulates the establishment of latency. *J. Virol.* 1997; 71(7):5432–5440. [PubMed: 9188615]
98. Sawtell NM, Thompson RL. Herpes simplex virus type 1 latency-associated transcription unit promotes anatomical site-dependent establishment and reactivation from latency. *J. Virol.* 1992; 66(4):2157–2169. [PubMed: 1312626]
99. Perng GC, Slanina SM, Yukht A, Ghiasi H, Nesburn AB, Wechsler SL. The latency-associated transcript gene enhances establishment of herpes simplex virus type 1 latency in rabbits. *J. Virol.* 2000; 74(4):1885–1891. [PubMed: 10644361]
100. Cook SD, Hill JM. Herpes simplex virus: molecular biology and the possibility of corneal latency. *Surv. Ophthalmol.* 1991; 36(2):140–148. [PubMed: 1659745]
101. O'Brien WJ, Taylor JL. The isolation of herpes simplex virus from rabbit corneas during latency. *Invest. Ophthalmol. Vis. Sci.* 1989; 30(30):357–364. [PubMed: 2538402]
102. Kennedy DP, Clement C, Arceneaux RL, Bhattacharjee PS, Huq TS, Hill JM. Ocular herpes simplex virus type 1: is the cornea a reservoir for viral latency or a fast pit stop? *Cornea.* 2011; 30(3):251–259. [PubMed: 21304287]
103. Penfold MET, Armati P, Cunningham AL. Axonal transport of herpes simplex virions to epidermal cells: evidence for a specialized mode of virus transport and assembly. *Proc. Natl Acad. Sci. USA.* 1994; 91(14):6529–6533. [PubMed: 7517552]
104. Tomishima MJ, Smith GA, Enquist LW. Sorting and transport of alpha herpesviruses in axons. *Traffic.* 2001; 2(7):429–436. [PubMed: 11422937]
105. Sandri-Goldin RM. Replication of the herpes simplex virus genome: does it really go around in circles? *Proc. Natl Acad. Sci. USA.* 2003; 100(13):7428–7429. [PubMed: 12810944]
106. Hill JM, Gebhardt BM, Wen RJ, et al. Quantitation of herpes simplex virus type 1 DNA and latency-associated transcripts in rabbit trigeminal ganglia demonstrates a stable reservoir of viral nucleic acids during latency. *J. Virol.* 1996; 70(5):3137–3141. [PubMed: 8627793] [Documented for the first time in an animal model that HSV-1 DNA and latency-associated transcript were a stable reservoir in the trigeminal ganglia over a long period of time.]
107. Bloom DC. HSV LAT and neuronal survival. *Int. Rev. Immunol.* 2004; 23(1–2):187–198. [PubMed: 14690860]
108. Bloom, DC. HSV-1 latency and the roles of the LATs.. In: Sandri-Goldin, RM., editor. *Alpha Herpesviruses: Molecular and Cellular Biology*. First Edition. Caister Academic Press; Norwich, UK: 2006. p. 325-342.
109. Rodahl E, Haarr L. Analysis of the 2-kilobase latency-associated transcript expressed in PC12 cells productively infected with herpes simplex virus type 1: Evidence for a stable, nonlinear structure. *J. Virol.* 1997; 71(2):1703–1707. [PubMed: 8995704]
110. Wu TT, Su YH, Block TM, Taylor JM. Evidence that two latency-associated transcripts of herpes simplex virus type 1 are nonlinear. *J. Virol.* 1996; 70(9):5962–5967. [PubMed: 8709218]
111. Berthomme H, Lokensgard J, Yang L, Margolis T, Feldman LT. Evidence for a bidirectional element located downstream from the herpes simplex virus type 1 latency-associated promoter that increases its activity during latency. *J. Virol.* 2000; 74(8):3613–3622. [PubMed: 10729137]

112. Lokensgard JR, Bloom DC, Dobson AT, Feldman LT. Long-term promoter activity during herpes simplex virus latency. *J. Virol.* 1994; 68(11):7148–7158. [PubMed: 7933097]
113. Margolis TP, Bloom DC, Dobson AT, Feldman LT, Stevens JG. Decreased reporter gene expression during latent infection with HSV LAT promoter constructs. *Virology.* 1993; 197(2): 585–592. [PubMed: 8249281]
114. Drolet BS, Perng GC, Cohen J, et al. The region of the herpes simplex virus type 1 LAT gene involved in spontaneous reactivation does not encode a functional protein. *Virology.* 1998; 242(1):221–232. [PubMed: 9501054]
115. Naito J, Mukerjee R, Mott KR, et al. Identification of a protein encoded in the herpes simplex virus type 1 latency associated transcript promoter region. *Virus Res.* 2005; 108(1–2):101–110. [PubMed: 15681060]
116. Thomas SK, Gough G, Latchman DS, Coffin RS. Herpes simplex virus latency-associated transcript encodes a protein which greatly enhances virus growth, can compensate for deficiencies in immediate-early gene expression, and is likely to function during reactivation from virus latency. *J. Virol.* 1999; 73(8):6618–6625. [PubMed: 10400759]
117. Thomas SK, Lilley CE, Latchman DS, Coffin RS. A protein encoded by the herpes simplex virus (HSV) type 1 2-kilobase latency-associated transcript is phosphorylated, localized to the nucleus, and overcomes the repression of expression from exogenous promoters when inserted into the quiescent HSV genome. *J. Virol.* 2002; 76(8):4056–4067. [PubMed: 11907244]
118. Randall G, Lagunoff M, Roizman B. The product of ORF O located within the domain of herpes simplex virus 1 genome transcribed during latent infection binds to and inhibits *in vitro* binding of infected cell protein 4 to its cognate DNA site. *Proc. Natl Acad. Sci. USA.* 1997; 94(19): 10379–10384. [PubMed: 9294219]
119. Henderson G, Jaber T, Carpenter D, Wechsler SL, Jones C. Identification of herpes simplex virus type 1 proteins encoded within the first 1.5 kb of the latency-associated transcript. *J. Neurovirol.* 2009; 15(5–6):439–448. [PubMed: 20175695]
120. Lagunoff M, Roizman B. Expression of a herpes simplex virus 1 open reading frame antisense to the gamma₁34. 5 gene and transcribed by an RNA 3' coterminal with the unspliced latency-associated transcript. *J. Virol.* 1994; 68(9):6021–6028. [PubMed: 8057477]
121. Lock M, Miller C, Fraser NW. Analysis of protein expression from within the region encoding the 2.0-kilobase latency-associated transcript of herpes simplex virus type 1. *J. Virol.* 2001; 75(7):3413–3426. [PubMed: 11238867]
122. Batchelor AH, O'Hare P. Regulation and cell-type-specific activity of a promoter located upstream of the latency-associated transcript of herpes simplex virus type 1. *J. Virol.* 1990; 64(7):3269–3279. [PubMed: 2161941]
123. Batchelor AH, O'Hare P. Localization of *cis*-acting sequence requirements in the promoter of the latency-associated transcript of herpes simplex virus type 1 required for cell-type-specific activity. *J. Virol.* 1992; 66(6):3573–3582. [PubMed: 1316469]
124. Batchelor AH, Wilcox KW, O'Hare P. Binding and repression of the latency-associated promoter of herpes simplex virus by the immediate early 175K protein. *J. Gen. Virol.* 1994; 75(Pt 4):753–767. [PubMed: 8151293]
125. Zwaagstra JC, Ghiasi H, Slanina SM, et al. Activity of herpes simplex virus type 1 latency-associated transcript (LAT) promoter in neuron-derived cells: Evidence for neuron specificity and for a large LAT transcript. *J. Virol.* 1990; 64(10):5019–5028. [PubMed: 2168984]
126. Perng G-C, Maguen B, Jin L, et al. A novel herpes simplex virus type 1 transcript (AL-RNA) antisense to the 5' end of the latency-associated transcript produces a protein in infected rabbits. *J. Virol.* 2002; 76(16):8003–8010. [PubMed: 12134005]
127. Jaber T, Henderson G, Li S, et al. Identification of a novel herpes simplex virus type 1 transcript and protein (AL3) expressed during latency. *J. Gen. Virol.* 2009; 90(Pt 10):2342–2352. [PubMed: 19570955]
128. Peng W, Vitvitskaia O, Carpenter D, Wechsler SL, Jones C. Identification of two small RNAs within the first 1.5-kb of the herpes simplex virus type 1-encoded latency-associated transcript 34. *J. Neurovirol.* 2008; 14(1):41–52. [PubMed: 18300074]

129. Bloom DC, Stevens JG, Hill JM, Tran RK. Mutagenesis of a cAMP response element within the latency-associated transcript promoter of HSV-1 reduces adrenergic reactivation. *Virology*. 1997; 236(1):202–207. [PubMed: 9299632]
130. Leib DA, Nadeau KC, Rundle SA, Schaffer PA. The promoter of the latency-associated transcripts of herpes simplex virus type 1 contains a functional cAMP-response element: Role of the latency-associated transcripts and cAMP in reactivation of viral latency. *Proc. Natl Acad. Sci. USA*. 1991; 88(1):48–52. [PubMed: 1846042]
131. Kenny JJ, Krebs FC, Hartle HT, et al. Identification of a second ATF/CREB-like element in the herpes simplex virus type 1 (HSV-1) latency-associated transcript (LAT) promoter. *Virology*. 1994; 200(1):220–235. [PubMed: 8128624]
132. Millhouse S, Kenny JJ, Quinn PG, Lee V, Wigdahl B. ATF/CREB elements in the herpes simplex virus type 1 latency-associated transcript promoter interact with members of the ATF/CREB and AP-1 transcription factor families. *J. Biomed. Sci.* 1998; 5(6):451–464. [PubMed: 9845850]
133. Marquart ME, Zheng X, Tran RK, Thompson HW, Bloom DC, Hill JM. A cAMP response element within the latency-associated transcript promoter of HSV-1 facilitates induced ocular reactivation in a mouse hyperthermia model. *Virology*. 2001; 284(1):62–69. [PubMed: 11352668]
134. Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. *Nature*. 2008; 454(7205):780–783. [PubMed: 18596690] [Identified microRNA that is expressed by HSV-1 during latency.]
135. Cui C, Griffiths A, Li G, et al. Prediction and identification of herpes simplex virus 1-encoded microRNAs. *J. Virol.* 2006; 80(11):5499–5508. [PubMed: 16699030]
136. Umbach JL, Nagel MA, Cohrs RJ, Gilden DH, Cullen BR. Analysis of human α -herpesvirus microRNA expression in latently infected human trigeminal ganglia. *J. Virol.* 2009; 83(20):10677–10683. [PubMed: 19656888]
137. Umbach JL, Cullen BR. The role of RNAi and microRNAs in animal virus replication and antiviral immunity. *Genes Dev.* 2009; 23(10):1151–1164. [PubMed: 19451215]
138. Stevens JG, Cook ML. Latent herpes simplex virus in spinal ganglia of mice. *Science*. 1971; 173(999):843–845. [PubMed: 4328483]
139. Pesola JM, Zhu J, Knipe DM, Coen DM. Herpes simplex virus 1 immediate-early and early gene expression during reactivation from latency under conditions that prevent infectious virus production. *J. Virol.* 2005; 79(23):14516–14525. Erratum appears in *J. Virol.* 80(12), 6196 (2006). [PubMed: 16282451]
140. Kwon BS, Gangarosa LP, Burch KD, deBack J, Hill JM. Induction of ocular herpes simplex virus shedding by iontophoresis of epinephrine into rabbit cornea. *Invest. Ophthalmol. Vis. Sci.* 1981; 21(3):442–449. [PubMed: 6268564]
141. Hill JM, Shimomura Y, Dudley JB, et al. Timolol induces HSV-1 ocular shedding in the latently infected rabbit. *Invest. Ophthalmol. Vis. Sci.* 1987; 28(3):585–590. [PubMed: 3030959]
142. Berman EJ, Hill JM. Spontaneous ocular shedding of HSV-1 in latently infected rabbits. *Invest. Ophthalmol. Vis. Sci.* 1985; 26(4):587–590. [PubMed: 2984140]
143. Hill JM, Rayfield MA, Haruta Y. Strain specificity of spontaneous and adrenergically induced HSV-1 ocular reactivation in latently infected rabbits. *Curr. Eye Res.* 1987; 6(1):91–97. [PubMed: 3030660]
144. Hill JM, Sedarati F, Javier RT, Wagner EK, Stevens JG. Herpes simplex virus latent phase transcription facilitates *in vivo* reactivation. *Virology*. 1990; 174(1):117–125. [PubMed: 2152989]
145. Leib DA, Bogard CL, Kosz-Vnenchak M, et al. A deletion mutant of the latency-associated transcript of herpes simplex virus type 1 reactivates from the latent state with reduced frequency. *J. Virol.* 1989; 63(7):2893–2900. [PubMed: 2542601]
146. Perng GC, Ghiasi H, Slanina SM, Nesburn AB, Wechsler SL. The spontaneous reactivation function of the herpes simplex virus type 1 LAT gene resides completely within the first 1.5 kilobases of the 8.3-kilobase primary transcript. *J. Virol.* 1996; 70(2):976–984. [PubMed: 8551638]

147. Trousdale MD, Steiner I, Spivack JG, et al. *In vivo* and *in vitro* reactivation impairment of a herpes simplex virus type 1 latency-associated transcript variant in a rabbit eye model. *J. Virol.* 1991; 65(12):6989–6993. [PubMed: 1658388]
148. Steiner I, Spivack JG, Lirette RP, et al. Herpes simplex virus type 1 latency-associated transcripts are evidently not essential for latent infection. *EMBO J.* 1989; 8(2):505–511. [PubMed: 2542019]
149. Thompson RL, Sawtell NM. Herpes simplex virus type 1 latency-associated transcript gene promotes neuronal survival. *J. Virol.* 2001; 75(14):6660–6675. [PubMed: 11413333]
150. Roizman B. The checkpoints of viral gene expression in productive and latent infection: the role of the HDAC/CoREST/LSD1/REST repressor complex. *J. Virol.* 2011; 85(15):7474–7482. [PubMed: 21450817]
151. Kristie TM, Liang Y, Vogel JL. Control of alpha-herpesvirus IE gene expression by HCF-1 coupled chromatin modification activities. *Biochim. Biophys. Acta.* 2010; 1799(3–4):257–265. [PubMed: 19682612]
152. Neumann DM, Bhattacharjee PS, Giordani NV, Bloom DC, Hill JM. *In vivo* changes in the patterns of chromatin structure associated with the latent herpes simplex virus type 1 genome in mouse trigeminal ganglia can be detected at early times after butyrate treatment. *J. Virol.* 2007; 81(23):13248–13253. [PubMed: 17881451]
153. Perng G-C, Jones C, Ciacci-Zanella J, et al. Virus-induced neuronal apoptosis blocked by the herpes simplex virus latency-associated transcript. *Science.* 2000; 287(5457):1500–1503. [PubMed: 10688801]
154. Hamza MA, Higgins DM, Feldman LT, Ruyechan WT. The latency-associated transcript of herpes simplex virus type 1 promotes survival and stimulates axonal regeneration in sympathetic and trigeminal neurons. *J. Neurovirol.* 2007; 13(1):56–66. [PubMed: 17454449]
155. Branco FJ, Fraser NW. Herpes simplex virus type 1 latency-associated transcript expression protects trigeminal ganglion neurons from apoptosis. *J. Virol.* 2005; 79(14):9019–9025. [PubMed: 15994795]
156. Jin L, Peng W, Perng G-C, et al. Identification of herpes simplex virus type 1 latency-associated transcript sequences that both inhibit apoptosis and enhance the spontaneous reactivation phenotype. *J. Virol.* 2003; 77(11):6556–6561. [PubMed: 12743314]
157. Henderson G, Peng W, Jin L, et al. Regulation of caspase 8- and caspase 9-induced apoptosis by the herpes simplex virus type 1 latency-associated transcript. *J. Neurovirol.* 2002; 8(Suppl. 2): 103–111. [PubMed: 12491160]
158. Ahmed M, Fraser NW. Herpes simplex virus type 1 2-kilobase latency-associated transcript intron associates with ribosomal proteins and splicing factors. *J. Virol.* 2001; 75(24):12070–12080. [PubMed: 11711597]
159. Carpenter D, Henderson G, Hsiang C, et al. Introducing point mutations into the ATGs of the putative open reading frames of the HSV-1 gene encoding the latency associated transcript (LAT) reduces its anti-apoptosis activity. *Microb. Pathog.* 2008; 44(2):98–102. [PubMed: 17900852]
160. Perng GC, Slanina SM, Yukht A, et al. A herpes simplex virus type 1 latency-associated transcript mutant with increased virulence and reduced spontaneous reactivation. *J. Virol.* 1999; 73(2):920–929. [PubMed: 9882292]
161. Garber DA, Schaffer PA, Knipe DM. A LAT-associated function reduces productive-cycle gene expression during acute infection of murine sensory neurons with herpes simplex virus type 1. *J. Virol.* 1997; 71(8):5885–5893. [PubMed: 9223478]
162. Smith JS, Robinson NJ. Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review. *J. Infect. Dis.* 2002; 186(Suppl. 1):S3–S28. [PubMed: 12353183]
163. Kaufman HE, Azcuy AM, Varnell ED, Sloop GD, Thompson HW, Hill JM. HSV-1 DNA in tears and saliva of normal adults. *Invest. Ophthalmol. Vis. Sci.* 2005; 46(1):241–247. [PubMed: 15623779] [Assessing tears and saliva for HSV-1 DNA twice a day over a 30-day period showed that >95% of human shed HSV-1 DNA.]
164. Kumar M, Hill JM, Clement C, Varnell ED, Thompson H, Kaufman HE. A double-blind placebo-controlled study to evaluate valacyclovir alone and with aspirin for asymptomatic HSV-1 DNA

- shedding in human tears and saliva. *Invest. Ophthalmol. Vis. Sci.* 2009; 50(12):5601–5608. [PubMed: 19608530]
165. Miller CS, Danaher RJ. Asymptomatic shedding of herpes simplex virus (HSV) in the oral cavity. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 2008; 105(1):43–50. [PubMed: 17703961]
 166. Abiko Y, Ikeda M, Hondo R. Secretion and dynamics of herpes simplex virus in tears and saliva of patients with Bell's palsy. *Otol. Neurotol.* 2002; 23(5):779–783. [PubMed: 12218634]
 167. Schmutzhard J, Merete Riedel H, Zwegyberg Wirgart B, Grillner L. Detection of herpes simplex virus type 1, herpes simplex virus type 2 and varicella-zoster virus in skin lesions. Comparison of real-time PCR, nested PCR and virus isolation. *J. Clin. Virol.* 2004; 29(2):120–126. [PubMed: 14747031]
 168. Tronstein E, Johnston C, Huang ML, et al. Genital shedding of herpes simplex virus among symptomatic and asymptomatic persons with HSV-2 infection. *JAMA.* 2011; 305(14):1441–1449. [PubMed: 21486977]
 169. Kaye SB, Madan N, Dowd TC, Hart CA, McCarthy K, Patterson A. Ocular shedding of herpes simplex virus. *Br. J. Ophthalmol.* 1990; 74(2):114–116. [PubMed: 2155653]
 170. Kaye SB, Baker K, Bonshek R, et al. Human herpesviruses in the cornea. *Br. J. Ophthalmol.* 2000; 84(6):563–571. [PubMed: 10837377]
 171. Espy MJ, Uhl JR, Mitchell PS, et al. Diagnosis of herpes simplex virus infections in the clinical laboratory by lightcycler PCR. *J. Clin. Microbiol.* 2000; 38(2):795–799. [PubMed: 10655387]
 172. Thouvenot D, Morfin F. Management of mucocutaneous herpes simplex virus infections in immunocompetent patients: signification and limits of antigen detection culture methods and antibody detection. *Ann. Dermatol. Venereol.* 2002; 129:609–619. [PubMed: 12122331]
 173. Espy MJ, Ross TK, Teo R, et al. Evaluation of LightCycler PCR for implementation of laboratory diagnosis of herpes simplex virus infection. *J. Clin. Microbiol.* 2000; 38(8):3116–3118. [PubMed: 10921993]
 174. Stranska R, Schuurman R, De Vos M, van Loon AM. Routine use of a highly automated and internally controlled real time PCR assay for the diagnosis of herpes simplex and varicella-zoster virus infections. *J. Clin. Virol.* 2004; 30(1):39–44. [PubMed: 15072752]
 175. Buddingh GJ, Schrum DI, Lanier J, Guidry D. Studies of the natural history of herpes simplex infections. *Pediatrics.* 1953; 11(6):595–610. [PubMed: 13073293]
 176. Kaufman HE, Brown DC, Ellison EM. Recurrent herpes in the rabbit and man. *Science.* 1967; 156:1628–1629. [PubMed: 4290539]
 177. Okinaga S. Shedding of herpes simplex virus type 1 into tears and saliva in healthy Japanese adults. *Kurume Med. J.* 2000; 47:273–277. [PubMed: 11197148]
 178. Posavad CM, Wald A, Hosken N, et al. T cell immunity to herpes simplex viruses in seronegative subjects: silent infection or acquired immunity? *J. Immunol.* 2003; 170(8):4380–4388. [PubMed: 12682275]
 179. Sawtell NM. The probability of *in vivo* reactivation of herpes simplex virus type 1 increases with the number of latently infected neurons in the ganglia. *J. Virol.* 1998; 72(8):6888–6892. [PubMed: 9658140]
 180. Richter ER, Dias JK, Gilbert JE, Atherton SS. Distribution of herpes simplex virus type 1 and varicella zoster virus in ganglia of the human head and neck. *J. Infect. Dis.* 2009; 200(12):1901–1906. [PubMed: 19919304]
 181. Pevenstein SR, Williams RK, McChesney D, Mont EK, Smialek JE, Straus SE. Quantitation of latent varicella-zoster virus and herpes simplex virus genomes in human trigeminal ganglia. *J. Virol.* 1999; 73(12):10514–10518. [PubMed: 10559370]
 182. Yamamoto Y, Hill JM. HSV-1 recovery from ocular tissues after viral inoculation into the superior cervical ganglion. *Invest. Ophthalmol. Vis. Sci.* 1986; 27(10):1447–1452. [PubMed: 3019918]
 183. Kaye SB, Lynas C, Patterson A, Risk JM, McCarthy K, Hart CA. Evidence for herpes simplex viral latency in the human cornea. *Br. J. Ophthalmol.* 1991; 75(4):195–200. [PubMed: 1850616]
 184. Openshaw H. Latency of herpes simplex virus in ocular tissue of mice. *Infect. Immun.* 1983; 39(2):960–962. [PubMed: 6299954]

185. Abghari SZ, Stulting R, Petrash J. Detection of herpes simplex virus type 1 latency-associated transcripts in corneal cells of inbred mice by *in situ* hybridization. *Cornea*. 1992; 11(5):433–438. [PubMed: 1330438]
186. Robert P-Y, Adenis J-P, Denis F, Alain S, Ranger-Rogez S. Herpes simplex virus DNA in corneal transplants: prospective study of 38 recipients. *J. Med. Virol.* 2003; 71(1):69–74. [PubMed: 12858411]
187. Abghari SZ, Stulting RD. Recovery of herpes simplex virus from ocular tissues of latently infected inbred mice. *Invest. Ophthalmol. Vis. Sci.* 1988; 29(2):239–243. [PubMed: 2828265]
188. Cook SD, Hill JM, Lynas C, Maitland NJ. Latency-associated transcripts in corneas and ganglia of HSV-1 infected rabbits. *Br. J. Ophthalmol.* 1991; 75(11):644–648. [PubMed: 1661140]
189. Openshaw H, McNeill JI, Lin XH, Niland J, Cantin EM. Herpes simplex virus DNA in normal corneas: persistence without viral shedding from ganglia. *J. Med. Virol.* 1995; 46(1):75–80. [PubMed: 7623011]
190. Upadhyay JM, Hill JM, Jemison M, Helmy MF, Kaufman HE. The effect of HSV multiplication rate on antiviral drug efficacy *in vitro*. *Antiviral Res.* 1991; 15(1):67–76. [PubMed: 1852150]
191. Koay PY, Lee WH, Figueiredo FC. Opinions on risk factors and management of corneal graft rejection in the United Kingdom. *Cornea*. 2005; 24(3):292–296. [PubMed: 15778601]
192. Maguire MG, Stark WJ, Gottsch JD, et al. Risk factors for corneal graft failure and rejection in the collaborative corneal transplantation studies. Collaborative Corneal Transplantation Studies Research Group. *Ophthalmology*. 1994; 101(9):1536–1547. [PubMed: 8090456]
193. Foster CS, Duncan J. Penetrating keratoplasty for herpes simplex keratitis. *Am. J. Ophthalmol.* 1981; 92(3):336–343. [PubMed: 7027796]
194. Beekhuis WH, Renardel de Lavalette JG, van Rij G, Schaap GJ. Therapeutic keratoplasty for active herpetic corneal disease: viral culture and prognosis. *Doc. Ophthalmol.* 1983; 55(1–2):31–35. [PubMed: 6301786]
195. Cosar CB, Sridhar MS, Cohen EJ, et al. Indications for penetrating keratoplasty and associated procedures, 1996–2000. *Cornea*. 2002; 21(2):48–151. [PubMed: 11805507]
196. Remeijer L, Duan R, van Dun JM, Wefers Bettink MA, Osterhaus AD, Verjans GM. Prevalence and clinical consequences of herpes simplex virus type 1 DNA in human cornea tissues. *J. Infect. Dis.* 2009; 200(1):11–19. [PubMed: 19476433]
197. Zheng X, Marquart ME, Loutsch JM, et al. HSV-1 migration in latently and naive rabbits after penetrating keratoplasty. *Invest. Ophthalmol. Vis. Sci.* 1999; 40(11):2490–2497. [PubMed: 10509641]
198. Remeijer L, Maertzdorf J, Doornenbal P, Verjans GM, Osterhaus AD. Herpes simplex virus 1 transmission through corneal transplantation [Letter]. *Lancet*. 2001; 357(9254):442. [PubMed: 11273067]
199. Rezende RA, Uchoa UBC, Raber IM, Rapuano CJ, Laibson PR, Cohen EJ. New onset of herpes simplex virus epithelial keratitis after penetrating keratoplasty. *Am. J. Ophthalmol.* 2004; 137(3):415–419. [PubMed: 15013862]
200. Hill JM, Clement C. Herpes simplex virus type 1 DNA in human corneas: what are the virological and clinical implications? *J. Infect. Dis.* 2009; 200(1):1–4. [PubMed: 19476431]
201. Kaufman, H.; Barron, BA.; McDonald, MB.; Kaufman, SC. *The Cornea*. Second Edition. Butterworth-Heinemann; Woburn, MA, USA: 2010.
202. Pepose JS, Keadle TL, Morrison LA. Ocular herpes simplex: changing epidemiology, emerging disease patterns, and the potential of vaccine prevention and therapy. *Am. J. Ophthalmol.* 2006; 141(3):547–557. [PubMed: 16490506]
203. Sawtell NM, Thompson RL. Rapid *in vivo* reactivation of herpes simplex virus in latently infected murine ganglionic neurons after transient hyperthermia. *J. Virol.* 1992; 66(4):2150–2156. [PubMed: 1312625]
204. Miller CS, Danaher RJ, Jacob RJ. Molecular aspects of herpes simplex virus 1 latency, reactivation and recurrence. *Crit. Rev. Oral Biol. Med.* 1998; 9(4):541–562. [PubMed: 9825226]
205. Kosz-Vnenchak M, Coen DM, Knipe DM. Restricted expression of herpes simplex virus lytic genes during establishment of latent infection by thymidine kinase-negative mutant viruses. *J. Virol.* 1990; 64(11):5396–5402. [PubMed: 2170678]

206. Tenser RB, Edris WA, Hay KA, de Galan BE. Expression of herpes simplex virus type 2 latency-associated transcript in neurons and nonneurons. *J. Virol.* 1991; 65(5):2745–2750. [PubMed: 1850049]
207. Bloom DC, Devi-Rao GB, Hill JM, Stevens JG, Wagner EK. Molecular analysis of herpes simplex virus type 1 during epinephrine-induced reactivation of latently infected rabbits *in vivo*. *J. Virol.* 1994; 68(3):1283–1292. [PubMed: 8107194]
208. Kriesel JD, Ricigliano J, Spruance SL, Garza HH Jr, Hill JM. Neuronal reactivation of herpes simplex virus may involve interleukin-6. *J. Neurovirol.* 1997; 3(6):441–448. [PubMed: 9475116]
209. Kriesel JD, Gebhardt BM, Hill JM, et al. Anti-interleukin-6 antibodies inhibit herpes simplex virus reactivation. *J. Infect. Dis.* 1997; 175(4):821–827. [PubMed: 9086136]
210. Kriesel JD, Araneo B, Petajan JP, Spruance SL, Stromatt S. Herpes labialis associated with recombinant human ciliary neurotrophic factor. *J. Infect. Dis.* 1994; 170(4):1046. [PubMed: 7930707]
211. Hill JM, Garza HH Jr, Helmy MF, et al. Nerve growth factor antibody stimulates reactivation of ocular herpes simplex virus type 1 in latently infected rabbits. *J. Neurovirol.* 1997; 3(3):206–211. [PubMed: 9200068]
212. Johnson, EM.; Manning, PT.; Wilcox, C. The biology of nerve growth factor *in vivo*. In: Ferrendelli, JA.; Collins, RC.; Johnson, EM., editors. *Neurobiology of Amino Acids, Peptides, and Tropic Factors*. Kluwer Academic Publishers; Hingham, MA, USA: 1988. p. 101–114.
213. Wilcox CL, Johnson EM Jr. Characterization of nerve growth factor-dependent herpes simplex virus latency in neurons *in vitro*. *J. Virol.* 1988; 62(2):393–399. [PubMed: 2826804]
214. Margolis TP, Elfman FL, Leib D, et al. Spontaneous reactivation of herpes simplex virus type 1 in latently infected murine sensory ganglia. *J. Virol.* 2007; 81(20):11069–11074. [PubMed: 17686862]
215. Feldman LT, Ellison AR, Voytek CC, Yang L, Krause P, Margolis TP. Spontaneous molecular reactivation of herpes simplex virus type 1 latency in mice. *Proc. Natl Acad. Sci. USA.* 2002; 99(2):978–983. [PubMed: 11773630]
216. Kaufman HE, Varnell ED, Thompson HW. Latanoprost increases the severity and recurrence of herpetic keratitis in the rabbit. *Am. J. Ophthalmol.* 1999; 127(5):531–536. [PubMed: 10334345]
217. Wand M, Gilbert CM, Liesegang TJ. Latanoprost and herpes simplex keratitis. *Am. J. Ophthalmol.* 1999; 127(5):602–604. [PubMed: 10334356]
218. Gebhardt BM, Varnell ED, Kaufman HE. Acetylsalicylic acid reduces viral shedding induced by thermal stress. *Curr. Eye Res.* 2004; 29(2–3):119–126. [PubMed: 15512958]
219. Gebhardt BM, Varnell ED, Kaufman HE. Inhibition of cyclooxygenase 2 synthesis suppresses *Herpes simplex* virus type 1 reactivation. *J. Ocul. Pharmacol. Ther.* 2005; 21(2):114–120. [PubMed: 15857277]
220. Cunningham AL, Diefenbach RJ, Miranda-Saksena M, et al. The cycle of human herpes simplex virus infection: virus transport and immune control. *J. Infect. Dis.* 2006; 194(Suppl. 1):S11–S18. [PubMed: 16921466]
221. Spruance SL. The natural history of recurrent oral-facial herpes simplex virus infection. *Semin. Dermatol.* 1992; 11(3):200–206. [PubMed: 1390034]
222. Spear PG, Roziman B. Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirion. *J. Virol.* 1972; 9:143–159. [PubMed: 4110104]
223. Remeijer L, Maertzdorf J, Buitenwerf J, Osterhaus AD, Verjans GM. Corneal herpes simplex virus type 1 superinfection in patients with recrudescence herpetic keratitis. *Invest. Ophthalmol. Vis. Sci.* 2002; 43(2):358–363. [PubMed: 11818377]
224. Pica F, Volpi A, Gaziano R, Garaci. Interferon-lambda in immunocompetent individuals with a history of recurrent herpes labialis. *Antivir. Ther.* 2010; 15(5):737–743. [PubMed: 20710055]
225. Herpetic Eye Disease Study Group. Predictors of recurrent herpes simplex virus keratitis. Herpetic Eye Disease Study Group. *Cornea.* 2001; 20(2):123–128. [PubMed: 11248812]
226. Herpetic Eye Disease Study Group. Psychological stress and other potential triggers for recurrences of herpes simplex virus eye infections. *Arch. Ophthalmol.* 2000; 118(12):1617–1624. [PubMed: 11115255]

227. Sakaoka H, Aomori T, Gouro T, Kumamoto Y. Demonstration of either endogenous recurrence or exogenous reinfection by restriction endonuclease cleavage and analysis of herpes simplex virus from patients with recrudescing genital herpes. *J. Med. Virol.* 1995; 46:387–396. [PubMed: 7595418]
228. Sucato G, Wald A, Wakabayashi E, Vieira J, Corey L. Evidence of latency and reactivation of both herpes simplex virus (HSV)-1 and HSV-2 in the genital region. *J. Infect. Dis.* 1998; 177(4): 1069–1072. [PubMed: 9534984]
229. Duan R, de Vries RD, Osterhaus AD, Remeijer L, Verjans GM. Acyclovir-resistant corneal HSV-1 isolates from patients with herpetic keratitis. *J. Infect. Dis.* 2008; 198(5):659–663. [PubMed: 18627246]
230. Duan R, de Vries RD, van Dun JM, et al. Acyclovir susceptibility and genetic characteristics of sequential herpes simplex virus type 1 corneal isolates from patients with recurrent herpetic keratitis. *J. Infect. Dis.* 2009; 200(9):1402–1414. [PubMed: 19795980]
231. Kaufman H, Martola E-L, Dohlman C. Use of 5-iodo-2'-deoxyuridine (IDU) in treatment of herpes simplex keratitis. *Arch. Ophthalmol.* 1962; 68:235–239. [PubMed: 14454436]
232. Wilhelmus KR. Therapeutic interventions for herpes simplex virus epithelial keratitis. *Cochrane Database Syst. Rev.* 2007;CD002898. [PubMed: 17253484]
233. Sozen E, Avunduk AM, Akyol N. Comparison of efficacy of oral valacyclovir and topical acyclovir in the treatment of herpes simplex keratitis: a randomized clinical trial. *Chemotherapy.* 2006; 52(1):29–31. [PubMed: 16340195]
234. Hoh HB, Hurley C, Claoue C, et al. Randomised trial of ganciclovir and acyclovir in the treatment of herpes simplex dendritic keratitis: a multicentre study. *Br. J. Ophthalmol.* 1996; 80(2):140–143. [PubMed: 8814744]
235. Miserocchi E, Modorati G, Galli L, Rama P. Efficacy of valacyclovir vs acyclovir for the prevention of recurrent herpes simplex virus eye disease: a pilot study. *Am. J. Ophthalmol.* 2007; 144(4):547–551. [PubMed: 17692271]
236. Nunes OS, Pereira RS. Regression of herpes viral infection symptoms using melatonin and SB-73: comparison with acyclovir 3. *J. Pineal Res.* 2008; 44(4):373–378. [PubMed: 18410585]
237. Herpetic Eye Disease Study Group. Oral acyclovir for herpes simplex virus eye disease. Effect on prevention of epithelial keratitis and stromal keratitis. *Arch. Ophthalmol.* 2000; 118(8):1030–1036. [PubMed: 10922194]
238. Quan D, Hammack BN, Kittelson J, Gilden DH. Improvement of postherpetic neuralgia after treatment with intravenous acyclovir followed by oral valacyclovir. *Arch. Neurol.* 2006; 63(7): 940–942. [PubMed: 16682531]
239. Pavan-Langston D, Lass J, Hettinger M, Udell I. Acyclovir and vidarabine in the treatment of ulcerative herpes simplex keratitis. *Am. J. Ophthalmol.* 1981; 92(6):829–835. [PubMed: 7032303]
240. Spruance SL, Jones TM, Blatter MM, et al. High-dose, short-duration, early valacyclovir therapy for episodic treatment of cold sores: results of two randomized, placebo-controlled, multicenter studies. *Antimicrob. Agents Chemother.* 2003; 47(3):1072–1080. [PubMed: 12604544]
241. Dhaliwal DK, Romanowski EG, Yates KA, et al. Valacyclovir inhibition of recovery of ocular herpes simplex virus type 1 after experimental reactivation by laser *in situ* keratomileusis. *J. Cataract Refract. Surg.* 2001; 27(8):1288–1293. [PubMed: 11524202]
242. Hill JM, Kwon B, Burch KD, et al. Acyclovir and vidarabine monophosphate: comparison of iontophoretic and intravenous administration for the treatment of HSV-1 stromal keratitis. *Am. J. Med.* 1982; 73(1A):300–304. [PubMed: 6179415]
243. Asbell PA. Valacyclovir for the prevention of recurrent herpes simplex virus eye disease after excimer laser photokeratectomy. *Trans. Am. Ophthalmol. Soc.* 2000; 98:285–303. [PubMed: 11190029]
244. Anand BS, Hill JM, Dey S, et al. In vivo antiviral efficacy of a dipeptide acyclovir prodrug, val-valacyclovir, against HSV-1 epithelial and stromal keratitis in the rabbit eye model. *Invest. Ophthalmol. Vis. Sci.* 2003; 44(6):2529–2534. [PubMed: 12766053]

245. Majumdar S, Nashed YE, Patel K, et al. Dipeptide monoester ganciclovir prodrugs for treating HSV-1-induced corneal epithelial and stromal keratitis: *in vitro* and *in vivo* evaluations. *J. Ocul. Pharmacol. Ther.* 2005; 21(6):463–474. [PubMed: 16386088]
246. Kaufman HE, Varnell ED, Thompson HW. Trifluridine, cidofovir, and penciclovir in the treatment of experimental herpetic keratitis. *Arch. Ophthalmol.* 1998; 116(6):777–778. [PubMed: 9639447]
247. Kaufman HE, Varnell ED, Gebhardt BM, et al. Efficacy of a helicase-primase inhibitor in animal models of ocular herpes simplex virus type 1 infection. *J. Ocul. Pharmacol. Ther.* 2008; 24(1): 34–42. [PubMed: 18201137]
248. Kumar M, Kaufman HE, Clement C, et al. Effect of high versus low oral doses of valacyclovir on herpes simplex virus-1 DNA shedding into tears of latently infected rabbits. *Invest. Ophthalmol. Vis. Sci.* 2010; 51(9):4703–4706. [PubMed: 20393107]
249. Gebhardt BM, Kaufman HE, Hill JM. Effect of acyclovir on thermal stress-induced herpesvirus reactivation. *Curr. Eye Res.* 2004; 29(2–3):137–144. [PubMed: 15512960]
250. Johnson TP, Frey R, Modugno M, Brennan TP, Margulies BJ. Development of an aciclovir implant for the effective long-term control of herpes simplex virus type-1 infection in Vero cells and in experimentally infected SKH-1 mice. *Int. J. Antimicrob. Agents.* 2007; 30(5):428–435. [PubMed: 17851051]
251. LeBlanc RA, Pesnicak L, Godleski M, Straus SE. Treatment of HSV-1 infection with immunoglobulin or acyclovir: comparison of their effects on viral spread, latency, and reactivation. *Virology.* 1999; 262(1):230–236. [PubMed: 10489356]
252. Loutsch JM, Sainz B Jr, Marquart ME, et al. Effect of famciclovir on herpes simplex virus type 1 corneal disease and establishment of latency in rabbits. *Antimicrob. Agents Chemother.* 2001; 45(7):2044–2053. [PubMed: 11408221]
253. Brandt CR, Akkarawongsa R, Altmann S, et al. Evaluation of a theta-defensin in a murine model of herpes simplex virus type 1 keratitis. *Invest. Ophthalmol. Vis. Sci.* 2007; 48(11):5118–5124. [PubMed: 17962464]
254. Yoon KC, Heo H, Kang IS, Lee MC, Kim KK, Park SH, Cho KO. Effect of topical cyclosporin A on herpetic stromal keratitis in a mouse model. *Cornea.* 2008; 27(4):454–460. [PubMed: 18434850]
255. Wiederrecht G, Lam E, Hung S, Martin M, Sigal N. The mechanism of action of FK-506 and cyclosporin A. *Ann. NY Acad. Sci.* 1993; 696:9–19. [PubMed: 7509138]
256. Knickelbein JE, Hendricks RL, Charukamnoetkanok P. Management of herpes simplex virus stromal keratitis: an evidence-based review. *Surv. Ophthalmol.* 2009; 54(2):226–234. [PubMed: 19298901]
257. Van Rooij J, Rijneveld WJ, Remeijer LJ, Beekhuis WH. A retrospective study on the effectiveness of oral acyclovir to prevent herpes simplex recurrence in corneal grafts. *Eur. J. Ophthalmol.* 1995; 5(4):214–218. [PubMed: 8963156]
258. Gupta R, Wald A, Krantz E, et al. Valacyclovir and acyclovir for suppression of shedding of herpes simplex virus in the genital tract. *J. Infect. Dis.* 2004; 190(8):1374–1381. [PubMed: 15378428]
259. Wachsman M, Aurelian L, Burnett JW. The prophylactic use of cyclooxygenase inhibitors in recurrent herpes simplex infections. *Br. J. Dermatol.* 1990; 123(3):375–380. [PubMed: 2119789]
260. Yin J, Huang Z, Xia Y, et al. Lornoxicam suppresses recurrent herpetic stromal keratitis through down-regulation of nuclear factor-kappa β : an experimental study in mice. *Mol. Vis.* 2009; 15:1252–1259. [PubMed: 19547717]
261. Hoglund M, Ljungman P, Weller S. Comparable aciclovir exposures produced by oral valacyclovir and intravenous aciclovir in immunocompromised cancer patients. *J. Antimicrob. Chemother.* 2001; 47(6):855–861. [PubMed: 11389118]
262. Ormrod D, Scott LJ, Perry CM. Valacyclovir: a review of its long term utility in the management of genital herpes simplex virus and cytomegalovirus infections. *Drugs.* 2000; 59(4):839–863. [PubMed: 10804039]
263. Asahi T, Tsutsui M, Wakasugi M, et al. Valacyclovir neurotoxicity: clinical experience and review of the literature. *Eur. J. Neurol.* 2009; 16(4):457–460. [PubMed: 19187258]

264. Colin J. Ganciclovir ophthalmic gel, 0.15%: a valuable tool for treating ocular herpes. *Clin. Ophthalmol.* 2007; 1(4):441–453. [PubMed: 19668521]
265. Wellings PC, Awdry PN, Bors FH, Jones BR, Brown DC, Kaufman HE. Clinical evaluation of trifluorothymidine in the treatment of herpes simplex corneal ulcers. *Am. J. Ophthalmol.* 1972; 73(6):932–942. [PubMed: 4555825]
266. Hobden JA, Kumar M, Kaufman HE, et al. In vitro synergism of trifluorothymidine and ganciclovir against HSV-1. *Invest. Ophthalmol. Vis. Sci.* 2011; 52(2):830–833. [PubMed: 20861476]
267. Yin, MT.; James, CM.; Brust, HVT.; Hammer, SM. *Clinical Virology*. 3rd Edition. ASM Press; Washington DC, USA: 2009. p. 217-264.
268. Birch CJ, Tyssen DP, Tachedjian G, et al. Clinical effects and *in vitro* studies of trifluorothymidine combined with interferon-alpha for treatment of drug-resistant and -sensitive herpes simplex virus infections. *J. Infect. Dis.* 1992; 166(1):108–112. [PubMed: 1318909]
269. Marcelletti JF. Synergistic inhibition of herpesvirus replication by docosanol and antiviral nucleoside analogs. *Antiviral Res.* 2002; 56(2):153–166. [PubMed: 12367721]
270. Bijnsdorp IV, Kruyt FA, Gokoel S, Fukushima M, Peters GJ. Synergistic interaction between trifluorothymidine and docetaxel is sequence dependent. *Cancer Sci.* 2008; 99(11):2302–2308. [PubMed: 18957056]
271. Shi W, Chen M, Xie L. Amniotic membrane transplantation combined with antiviral and steroid therapy for herpes necrotizing stromal keratitis. *Ophthalmology.* 2007; 114(8):1476–1481. [PubMed: 17363059]
272. Bulet P, Stocklin R, Menin L. Anti-microbial peptides: from invertebrates to vertebrates. *Immunol. Rev.* 2004; 198:169–184. [PubMed: 15199962]
273. Zanetti M. The role of cathelicidins in the innate host defenses of mammals. *Curr. Issues Mol. Biol.* 2005; 7(2):179–196. [PubMed: 16053249]
274. Sahl HG, Pag U, Bonness S, Wagner S, Antcheva N, Tossi A. Mammalian defensins: structures and mechanism of antibiotic activity. *J. Leukoc. Biol.* 2005; 77(4):466–475. [PubMed: 15582982]
275. Bultmann H, Teuton J, Brandt CR. Addition of a C-terminal cysteine improves the anti-herpes simplex virus activity of a peptide containing the human immunodeficiency virus type 1 TAT protein transduction domain. *Antimicrob. Agents Chemother.* 2007; 51(5):1596–1607. [PubMed: 17261627]
276. Akkarawongsa R, Pocaro NE, Case G, Kolb AW, Brandt CR. Multiple peptides homologous to herpes simplex virus type 1 glycoprotein B inhibit viral infection. *Antimicrob. Agents Chemother.* 2009; 53(3):987–996. [PubMed: 19104014]
277. Bultmann H, Brandt CR. Peptides containing membrane-transiting motifs inhibit virus entry. *J. Biol. Chem.* 2002; 277(39):36018–36023. [PubMed: 12130644]
278. Bultmann H, Busse JS, Brandt CR. Modified FGF4 signal peptide inhibits entry of herpes simplex virus type 1. *J. Virol.* 2001; 75(6):2634–2645. [PubMed: 11222686]
279. Garcia-Bassets I, Kwon YS, Telese F, et al. Histone methylation-dependent mechanisms impose ligand dependency for gene activation by nuclear receptors. *Cell.* 2007; 128(3):505–518. [PubMed: 17289570]
280. Metzger E, Wissmann M, Yin N, et al. LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature.* 2005; 437(7057):436–439. [PubMed: 16079795]
281. Perillo B, Ombra MN, Bertoni A, et al. DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. *Science.* 2008; 319(5860):202–206. [PubMed: 18187655]
282. Liang Y, Vogel JL, Narayanan A, Peng H, Kristie TM. Inhibition of the histone demethylase LSD1 blocks alpha-herpesvirus lytic replication and reactivation from latency. *Nat. Med.* 2009; 15(11):1312–1317. [PubMed: 19855399] [First report that a monoamine oxidase inhibitor could block *in vitro* neuronal reactivation of HSV-1.]

283. Pivetti-Pezzi P, Accorinti M, Colabelli-Gisoldi A, Pirraglia MP, Siranni MC. Herpes simplex virus vaccine in recurrent herpetic ocular infection. *Cornea*. 1999; 18(1):47–51. [PubMed: 9894936]
284. Bettahi I, Nesburn AB, Yoon S, et al. Protective immunity against ocular herpes infection and disease induced by highly immunogenic self-adjuvanting glycoprotein D lipopeptide vaccines. *Invest. Ophthalmol. Vis. Sci*. 2007; 48(10):4643–4653. [PubMed: 17898288]

Box 1. Mouse eye models of herpes simplex virus-1 acute, latent and recurrent infection**Advantages**

- Inbred strains readily available
- Transgenic and/or target replacement strains available but can be expensive
- Small size decreases the cost of rare and/or expensive drugs or chemicals
- Low cost of boarding
- Low cost of most inbred strains

Disadvantages

- Acute corneal lesions difficult to quantify and photograph
- Spontaneous recurrent lesions occur only rarely, if ever
- Induced corneal lesions are difficult to achieve and quantify
- Very small tear volume probably results in a decrease in the detection of infectious herpes simplex virus and herpes simplex virus DNA
- Small amounts of ocular and neural tissues reduce the number of assays and increase the number of mice needed per time point

Box 2. Rabbit eye model of herpes simplex virus-1 acute, latent and recurrent infections**Advantages**

- Acute corneal lesions easily quantified and photographed
- Spontaneous lesions can occur when rabbits are latent with a HPR
- Induced corneal lesions can be quantified when rabbits are latent with a HPR
- Large size eye and tear volume allows easy collection of tears
- Large body size provides more ocular and neural tissues

Disadvantages

- Inbred strains difficult to obtain and expensive
- Very limited transgenic and/or targeted replacement rabbits
- Inoculation with HPR (strains McKrae and Syn+) results in 50% mortality
- Corneal inoculation with HPR results in 5–10% of corneas with stromal opacity
- High cost of purchase and boarding

HPR: High phenotypic reactivator.

Box 3. Risk factors for recurrent ocular herpes simplex virus disease in patients with a history of active ocular herpes simplex virus disease during the previous year

■ Risk factors

- More previous episodes of stromal keratitis = increased risk of stromal keratitis recurrence (10x more likely)
- History of stromal keratitis even if treated with acyclovir = increased risk of stromal keratitis recurrence

■ Nonrisk factors

- Age
- Gender
- Race
- History of epithelial keratitis
- History of nonocular herpes simplex virus infection
- Season of the year
- Exogenous catalysts
- Psychogenic profile

Adapted with permission from [225].

Executive summary

- Herpes simplex virus (HSV)-1 is latent in the peripheral and central nervous system of most humans.
- Most humans shed HSV-1 DNA in the tears and saliva.
- The presence of latency-associated transcript is proof of HSV latency.
- The frequency and incidence of ocular herpes is low (estimated 2–5%).
- Studies in animal models have shown HSV-1 strains can be either high or low phenotypic reactivators.
- Two specific genetic host factors, ApoE $\epsilon 4/\epsilon 4$ and *C21orf91*, have been suggested to be risk factors for ocular herpes and cold sores, respectively. In general, antiherpetic chemotherapy is successful for a majority of cases. However, repeated reactivations cause serious ocular herpes that can be refractive to chemotherapy.

Conclusion

- For refractive ocular herpes there is a need to identify host factors that could justify complex chemotherapeutic protocols to successfully treat corneal disease to prevent irreversible stromal damage.
- Possible chemotherapeutic modulation could come from ApoE mimetic peptides that have been shown to demonstrate antiviral, anti-inflammatory and antineovascular activity.

Table 1

Herpes gene candidates on chromosome 21 related to recurrent herpes labialis.

Gene	Protein	Locus	Function/association
<i>USP25</i>	Ubiquitin-specific protease 25	21q11.2	Removes ubiquitin from tagged proteins
<i>BTG3</i>	B cell translocation gene	21q21.1	Tob/BTG1 family antiproliferative protein, abundant in neuroepithelium
<i>CXADR</i>	Coxsackievirus and adenovirus receptor	21q11.2	Confers susceptibility of cell cultures to coxsackievirus infections
<i>C21orf34</i>	Chromosome 21 ORF 34	21q21.1	Protein product with unknown function
<i>C21orf91</i> [†]	Chromosome 21 ORF 91	21q21.1	Protein product with unknown function
<i>CHODL</i>	Chondrolectin	21q11.2	Transmembrane protein found in muscle and spleen

ORF: Open reading frame.

Data taken from [46].

[†] Recent evidence identified this as a primary candidate gene for recurrent herpes labialis susceptibility [50].

Table 2

Summary of studies on the presence of herpes simplex virus-1 DNA in human trigeminal ganglia.

Year	Number of positive TG/total number of TG (%) in indicated group			Ref.
	Total	Male	Female	
2009	18/58 (31.0) [†]	NA	NA	[180] ^{†§}
2008	155/174 (89.1)	82/91 (90.1)	73/83 (88.0)	[14]
2000	23/34 (67.6)	16/26 (61.5)	7/8 (87.5)	[7]
1999	8/15 (53.3)	6/11 (54.5)	2/4 (50.0)	[181]
2007	24/34 (70.6)	14/24 (58.3)	10/10 (100)	[13]
2002	31/46 (67.4)	8/10 (80.0)	23/36 (63.9)	[6] [†]
1992	8/11 (72.7)	5/7 (71.4)	3/4 (75.0)	[10] [¶]
1993	79/109 (72.5)	NA	NA	[9] [#]
2001	30/43 (69.8)	15/21 (71.4)	15/22 (68.2)	[5] [¶]
2006	74/121 (61.2)	43/77 (55.8)	31/49 (63.3)	[11] ^{††}
1986	11/20 (55.0)	7/12 (58.3)	4/8 (50.0)	[8]

NA: Not applicable; TG: Trigeminal ganglia.

[†]Three of the positive TG samples were obtained from specimens where the gender was unknown.[†]TG from formalin-fixed cadavers were used.[§]Age and gender were unavailable for one subject.[¶]No fixative was used; TG were frozen at autopsy.[#]109 pairs of TG were used; each pair was analyzed as one unit after 48 h of digestion. Age and gender were not given for individual TG.^{††}TG were stored in 99.5% alcohol before DNA extraction.

Table 3

Distribution of herpes simplex virus-1 and varicella zoster virus DNA in the ganglia of the head and neck.

Human ganglia	HSV-1	VZV	Range of amount of ganglia available [†]
TG	18/58 (31%)	16/58 (28%)	500 mg–1.5 g
Nodose	23/58 (39%)	11/58 (19%)	0.10–0.25 g
SCG	25/60 (41%)	14/60 (23%)	~0.015 g (15 mg)
Otic	25/48 (52%)	11/48 (23%)	NA
PTG	15/50 (30%)	8/50 (16%)	0.35–0.55 g
Submandibular	14/47 (30%)	4/47 (8.5%)	0.02 g
Ciliary	18/58 (31%)	10/58 (17%)	0.35–0.75 g
Geniculate	12/36 (33%)	1/36 (2.8%)	0.50–0.70 g
Totals	150/415 (36%)	75/415 (18%)	

22/36 VZVDNA = 61%.

32/36 HSV-1 DNA = 89%

VZV DNA + HSV-1 DNA 36/36 = 100%.

HSV: Herpes simplex virus; NA: Not available; TG: Trigeminal ganglia; VZV: Varicella zoster virus.

Data taken from [180] and [ATHERTON SS, PERS. COMM.].

[†]Used only ~15 mg/tissue.

Table 4

High versus low phenotypic reactivators based on a rabbit model[†].

Characteristics	Low phenotypic reactivators	High phenotypic reactivators
Viral strain	Most HSV-1 strains including F, KOS, SC-16, Rodanas, McIntyre, CGA-3	Rare 17 Syn+ and McKrae
HSV-1 DNA copy numbers in the trigeminal ganglia	Variable = high phenotypic reactivators	Variable = low phenotypic reactivators
Spontaneous HSV-1 DNA shedding in saliva	Frequent; low copy numbers	Frequent; high copy numbers
Spontaneous HSV-1 DNA shedding in the tears	Frequent; low copy numbers	Frequent; high copy numbers
Spontaneous shedding in tears of infectious virus	None or very low	High and frequent
Spontaneous ocular lesions	None or very low	High and frequent

HSV: Herpes simplex virus.

Data taken from [66,143].

[†]Summary of characteristics of rabbits latent with high or low phenotypic reactivators of HSV-1.

Table 5

Summary of human studies using antiherpetics.

Antiviral(s)	Dose/frequency	Route	Clinical condition	Outcome	Adverse effects	Ref.
ACV vs VACV	One application 5× daily for 30 days 1000 mg 2× daily for 30 days	Topical, oral	HSV epithelial and stromal keratitis	Oral VACV was as effective (or even more effective) in the treatment of herpetic keratitis as the topical ACV ointment	None	[233]
GCV vs ACV	0.15% GCV gel 3% ACV gel 5× daily until the ulcer is healed; then 3× daily for 3 more days	Topical	Herpes simplex dendritic keratitis	Equally effective in ability to heal corneal ulceration	None	[234]
VACV vs VACV with aspirin	500 mg VACV once daily for 30 days 500 mg VACV once daily + 350 mg aspirin 2× daily for 30 days	Oral	Asymptomatic HSV-1 DNA shedding in tears and saliva	No correlation was found between HSV-1 shedding and VACV and VACV with aspirin treatment, the HSV-1 DNA copy number was not reduced by treatment	None	[164]
VACV vs ACV	500 mg VACV once daily 400 mg ACV 2× daily for 12 months	Oral	Recurrent ocular HSV disease over 12 months	Equally effective and well tolerated, recurrence rate equal	Nausea, headache	[235]
ACV vs SB73/melatonin	2.5 mg melatonin and 100 mg SB-73 once daily for 7 days 200 mg ACV 5× daily for 5 days	Oral	Recurrent HSV-1 or -2	SB-73/melatonin significantly reduced time to lesion healing and duration of pain/discomfort vs ACV	SB-73: somnolence ACV: gastrointestinal complaints/headache	[236]
ACV	400 mg 5× daily for 3 weeks	Oral	HSV-1 epithelial keratitis	For patients with HSV epithelial keratitis treated with topical trifluridine, no apparent benefit of a 3-week course of oral ACV in preventing HSV stromal keratitis or iritis was seen during the subsequent year	Diarrhea, nausea, headache	[237]
ACV	10 mg/kg 3× daily for 30 days	Intravenous	Varicella zoster	Reduced pain	None	[238]
ACV vs Vidarabine	3% ACV ointment 3% vidarabine ointment 5× daily for 14 days	Topical	HSK	No statistical difference	None	[239]
VACV	2 g VACV 2× daily × 1 day or 2 g VACV 2× daily × 1 day then 1 g VACV 2× daily × 1 day	Oral	Herpes labialis	1-day VACV treatment regimen is effective in reducing duration of episode	None	[240]

ACV: Acyclovir; GCV: Ganciclovir; HSK: Herpetic stromal keratitis; HSV: Herpes simplex virus; VACV: Valacyclovir.

Table 6

Summary of rabbit studies using antiherpetics.

Antiviral(s)	Route	Dose/frequency	Clinical conditions	Outcome	Adverse effects	Ref.
VACV	Intraperitoneal	100 mg/kg/day 200 mg/kg/day two injections daily for 7 days	Ocular HSV-1 shedding after LASIK	Systemic administration of VACV significantly reduced HSV-1 ocular shedding after LASIK	None	[241]
ACV vs ara-AMP	Iontophoresis and intravenous	0.5m Amp × 4 min with 3.4% ara-AMP and 5% ACV once daily for 5 days 50 mg/kg ACV intravenous 2× daily for 8 days	HSV-1 stromal keratitis	Iontophoresis of ACV or ara-AMP either alone or in combination with intravenous ACV decreased course of disease	None	[242]
VACV	Intraperitoneal	50, 100 or 150 mg/kg for 14 days	Reactivation of latent HSV-1 after excimer laser photorefractive keratectomy and de-epithelialization	Excimer laser treatment can trigger reactivation of ocular herpes disease and viral shedding in the latently infected rabbit. De-epithelialization alone is not sufficient to cause reactivation or viral shedding. Prophylaxis with intraperitoneal VACV decreases the recurrence rate in a dose-response fashion. At 150 mg/kg/day, there are no recurrences	None	[243]
Val-Val-ACV vs TFT	Topical	50 µl of 1% Val-Val-ACV and 1% TFT 5× daily starting 3 days after infection for 5 days	HSV-1 epithelial keratitis and stromal keratitis	1 % Val-Val-ACV was as effective as 1% TFT. The prodrug was also less cytotoxic than TFT	None	[244]
Dipeptide monoester GCV prodrugs vs TFT	Topical	50 µl at a concentration of 1% 5× daily beginning on PI day 3 and continuing for 5 consecutive days	HSV-1 epithelial and stromal keratitis	Val-Val-GCV demonstrates excellent corneal permeability and chemical stability, high aqueous solubility and substantial <i>in vivo</i> antiviral activity against the HSV-1. 1% Val-Val-GCV provided significantly better therapeutic activity than 1% TFT against HSV-1 epithelial keratitis and equivalent therapeutic activity against stromal keratitis	None	[245]
Trifluridine vs cidofovir vs penciclovir	Topical	1% trifluridine given either 2, 4 or 7× daily 0.2% cidofovir 2× daily 3% penciclovir ointment either 2× or 4× daily All starting PI day 3 continuing to day 9	HSV-1 epithelial keratitis	Cidofovir and trifluridine were equally effective against herpetic keratitis. Both trifluridine and cidofovir given as infrequently as once a day are effective for treating HSK, even with treatment beginning 72 h after infection when the lesions are well established. Topical penciclovir is slightly less effective than trifluridine or cidofovir and seems to offer no advantages. Although all treatments were effective in promoting the healing of herpetic keratitis, none prevented spontaneous recurrences, even while treatment was continuing, and all groups	None	[246]

Antiviral(s)	Route	Dose/frequency	Clinical conditions	Outcome	Adverse effects	Ref.
BAY 57-1293 (a helicase-primase inhibitor)	Oral	500 mg/kg suspension once daily on PI days 3-6 40 mg/kg/day VACV or 40 mg/kg/day VACV plus 40 mg/kg/day BAY 57-1293 or 40 mg/kg/day BAY 57-1293 in one dose per day on PI days 3-10	HSV-1 keratitis	Once-daily oral treatment with 500 mg/kg BAY 57-1293 was as effective as twice-daily topical treatment with trifluridine, no difference seen in the severity of keratitis in the animals receiving 40 mg/kg/day BAY 57-1293 alone or 40 mg/kg/day BAY 57-1293 plus 40 mg/kg/day VACV. Keratitis in the animals treated with 40 mg/kg/day BAY-1293 was significantly less severe, compared with the animals treated with VACV alone	None	[247]
BAY 57-1293 (a helicase-primase inhibitor)	Topical	One drop of 2% suspension of BAY 57-1293 in methylcellulose either 2 or 5× daily to each eye for 5 days, beginning 3 days PI	HSV-1 keratitis	Therapeutic effectiveness of the methylcellulose formulation, given 5× daily, was approximately the same as that of trifluridine. 2% suspensions of BAY 57-1293 in methylcellulose given 2× daily were less effective than the methylcellulose formulation given 5× daily	None	[247]
VACV	Oral	7 mg/kg VACV or 70 mg/kg VACV or 140 mg/kg VACV 2× daily starting on PI day 36 and continuing for 14 days	Asymptomatic shedding of HSV-1 DNA into tears	No observable toxicity in any group. The 70 mg/kg and 140 mg/kg doses of VACV significantly reduced the HSV-1 DNA copy numbers	None	[248]
FCV	Oral	60, 120, 250 or 500 mg of FCV 2× daily for 5 days starting on PI day 3	HSV-1 epithelial keratitis	Reduced the severity of corneal lesions, reduced the number of HSV-1 genomes in the TG, improved survival; not as effective as topical drops of TFT	None	[249]

ACV: Acyclovir; FCV: Fanciclovir; GCV: Ganciclovir; HSK: Herpetic stromal keratitis; HSV: Herpes simplex virus; LASIK: Laser-assisted in situ keratomileusis;

TFT: 5-trifluoromethyl-deoxyuridine; TG: Trigeminal ganglia; VACV: Valacyclovir.

Table 7

Summary of mouse studies using antiherpetics.

Antiviral(s)	Route	Dose/frequency	Clinical conditions	Outcome	Adverse effects	Ref.
ACV	Oral	3.5 mg/ml in drinking water for 4 days	Induction of reactivation of latent HSV-1 by hyperthermic stress	Oral ACV at the dose used in this study is effective in modestly reducing viral replication in peripheral tissues such as the eye but is not effective in inhibiting viral reactivation and viral DNA synthesis in the peripheral nervous system in mice subjected to induction of reactivation by hyperthermic stress	None	[250]
ACV	Subcutaneous implant	Average daily release of ACV was $1.07 \pm 0.760 \mu\text{g}$ over 63 days	Cutaneous HSV-1 reactivation via UV light	Implants protected HSV-1-infected mice from viral reactivation via UV light	Sunburn/flaking skin from UV exposure	[251]
ACV vs IgG	One time intraperitoneal injection 24 h after infection; ACV orally	0.5 ml IgG one time dose, 2 mg/ml ACV in drinking water for 7 days	Establishment, maintenance and reactivation from latency of HSV-1	IgG protects mice from HSV-1 infection essentially as well as ACV does. IgG permits higher levels of latent infection and subsequent <i>in vivo</i> reactivation	None	[252]
Apolipoprotein E	Topical	1 % apoEdp $5 \times$ daily vs 1% trifluorothymidine	Treatment of HSV-1	apoEdp treatment was equally effective as TFT in reducing the incidence and severity of HSK	None	[60]
BAY 57-1293 (a helicase-primase inhibitor)	Oral	Two oral doses of 50 mg/kg BAY 57-1293, one immediately after stress and one 12 h later	HSV-1 reactivation of latent infection and viral shedding	Suppressed reactivation in HSV-1-infected mice and reduced the viral load	None	[247]
RC-2	Topical	$5 \mu\text{l}$ 0.1% (wt/vol) RC-2, $4 \times$ daily for 7 days	Treatment and prevention of HSK	Applying RC-2 to the cornea in 2% methylcellulose before HSV-1 infection or incubating the peptide with HSV-1 greatly reduced the severity of the ocular infection, however, after viral infection, treatment with RC-2 did not reduce the severity	None	[253]
Cyclosporin A	Topical	0.01, 0.1 and 1% cyclosporin A, $4 \times$ daily	Treatment of clinical HSK of HSV-1	On days 7, 10 and 14 groups treated with >0.1 % cyclosporin A had decreased stromal haze and inflammation	None	[254]

ACV: Acyclovir; HSK: Herpetic stromal keratitis; HSV: Herpes simplex virus; RC: Retrocyclin; TFT: 5-trifluoromethyl-deoxyuridine.

Table 8

Effect of high versus low oral doses of valacyclovir on herpes simplex virus-1 DNA shedding into tears of latently infected rabbits.

Groups	Pretreatment				Post-treatment			
	Positive rabbits	Positive eyes	Positive swabs (%)	Mean copy number $\pm$ SEM	Positive rabbits	Positive eyes	Positive swabs (%)	Mean copy number $\pm$ SEM
Untreated	5/5	7/10	12/60 (19)	370 $\pm$ 70	4/5	5/10	19/100 (19)	232 $\pm$ 183
Placebo	5/5	5/10	19/60 (31)	569 $\pm$ 237	4/5	5/10	24/100 (24)	564 $\pm$ 186
7 mg/kg VCV	5/5	7/10	15/60 (25)	368 $\pm$ 86	4/5	5/10	20/100 (20)	518 $\pm$ 122
70 mg/kg VCV	5/5	6/10	15/60 (25)	408 $\pm$ 108	2/5	2/10	7/100 (7)	67 $\pm$ 63
140 mg/kg VCV	5/5	6/10	21/60 (33)	396 $\pm$ 91	1/5	2/10	7/100 (7)	13 $\pm$ 7

SEM: Standard error mean; VCV: Valacyclovir.

Data taken from [248].