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Progress in Retinal and Eye Research 25 (2006) 355–380
Progress in RETINAL AND EYE RESEARCH
www.elsevier.com/locate/prer

Herpes simplex keratitis

Stephen Kaye*, Anshoo Choudhary

St. Paul's Eye Unit and The Department of Medical Microbiology, Royal Liverpool University Hospital, Prescot Street, Liverpool L7 8XP, UK

Abstract

Herpes simplex keratitis (HSK) results from an infection with the herpes simplex virus type 1 (HSV-1) also known as human herpesvirus type 1 (HHV-1). Primary infection may involve an ocular or non-ocular site, following which latency might be established principally in the trigeminal ganglion but also in the cornea. During latency, the virus appears as a circular episome associated with histones with active transcription only from the region encoding the latency-associated transcript (LAT). The LAT region is implicated in neuronal survival, anti-apoptosis, virulence, suppression of transcription, establishment of and reactivation from latency. The initial keratitis may develop after infection through the "front door route" (entry into the ocular surface from droplet spread) or "back door route" (spread to the eye from a non-ocular site, principally the mouth). The initial ocular infection may be mild. Visual morbidity results from recurrent keratitis, which leads to corneal scarring, thinning and neovascularisation. Although, recurrent disease may potentially occur through anterograde axonal spread from the trigeminal ganglion to the cornea, recent evidence suggests that HSV-1 in the cornea may be another source of recurrent disease. The pathogenesis and severity of HSK is largely determined by an interaction between viral genes encoded by the strain of HSV-1 and the make up of the host's immune system. Herpetic stromal disease is due to the immune response to virus within the cornea and the ability of the strain to cause corneal stromal disease is correlated with its ability to induce corneal vascularisation. The pathogenesis of corneal scarring and vascularisation is uncertain but appears to be a complex interaction of various cytokines, chemokines and growth factors either brought in by inflammatory cells or produced locally in response to HSV-1 infection. Evidence now suggests that HSV-1 infection disrupts the normal equilibrium between angiogenic and anti-angiogenic stimuli leading to vascularisation. Thrombospondin 1 and 2, matricellular proteins, involved in wound healing are potent anti-angiogenic factors and appear to be one of the key players. Elucidating their roles in corneal scarring and vascularisation may lead to improved therapies for HSK.

Keywords: Herpes simplex virus; Herpes simplex keratitis; Corneal vascularisation; Latency associated transcripts

Contents

*Corresponding author. Tel.: +44 151 706 2134; fax +44 151 706 5861.
E-mail address: s.b.kaye@liverpool.ac.uk (S. Kaye).

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doi:10.1016/j.preteyeres.2006.05.001

0. Introduction

Herpes simplex keratitis (HSK) remains a major cause of visual morbidity. The incidence of herpetic ocular surface disease lies between 5.9 and $20.7/10^5$ of the population per year with a prevalence of $149/10^5$ in developed countries (Liesegang, 2001; Norn, 1970). Although there is less information available, the prevalence and incidence of herpetic eye disease in developing countries may be higher, affecting a younger population. The initial (not necessarily primary) sites of herpetic eye involvement usually manifest as a blepharitis, conjunctivitis or corneal epithelial keratitis. In contrast, recurrent disease manifests predominantly as an ulcerative and/or stromal keratitis. It is recurrent disease, however, which has the main impact on vision through corneal scarring, thinning and neovascularisation. Although predominantly unilateral, bilateral disease occurs in 1.3–12% of cases, occurs in a younger age group and tends to be more severe. This may be particularly acute for countries in the developing world, where a younger age group may be affected with more severe disease, compounded by the presence of malnutrition and other diseases as well as the lack of access to treatment. HSK is a result of infection predominantly with the herpes simplex virus type 1 (HSV-1) and reflects the interaction between viral and host factors. Detailed studies on the life cycle of HSV-1 and its behaviour within the host have led and continue to lead to significant advances into the pathogenesis and treatment of HSK.

1. Replication of HSV-1

1.1. Anatomy of HSV-1

HSV-1 is a large double-stranded DNA virus with a genome of approximately 152 kb (Fig. 1). It is an alphaherpesvius and belongs to the human herpesvirus (HHV) family, of which it is the first member, also referred to as HHV type 1 or HHV-1. The HSV-1 virion is 120–300 nm in size. It consists of an electron-opaque core containing the genome, a surrounding capsid, 100 nm in diameter, a tegument and an envelope. Capsid architecture is among the most characteristic feature of the herpesvirus family. It comprises 162 capsomers arranged to form an icosadeltahedron. The tegument is an amorphous structure surrounding the capsid that contains proteins and enzymes such as the important virion host shut-off (VHS) protein. The envelope consists of a lipid bilayer with about 12 embedded glycoproteins, which serve as attachment proteins (gB, gC, gD, gH), fusion proteins (gB), structural proteins and immune escape proteins (gC, gE, gI). Most evidence indicates that HSV-1 acquires its envelope from the host cell. As an enveloped virus, HSV-1 is sensitive to acid, solvents, detergents and drying. The genome is linearly divided into long and short regions of unique sequences, termed UL and US (UL = unique long; US = unique short), bounded by regions of internal and terminal repeats (Fig. 2). It is the variability in the number of these repeat regions, which leads to the variability in the

size of the genome (Knipe and Howley, 2001; Murray et al., 2002).

1.2. Viral replication

The virus attaches to cell receptors, following which fusion of the envelope occurs with the plasma membrane. The HSV-1 glycoproteins are intrinsic to this process. The capsid is then transported to the nuclear pores and DNA is released into the nucleus. After entry into the cell, there is a shutoff of host macromolecular synthesis, mediated by the VHS protein. Transcription, replication of viral DNA and assembly of new capsids takes place in the nucleus. Viral DNA is transcribed throughout the reproductive cycle by host RNA polymerase II and certain viral factors with a sequential coordination of gene expression (Sears and Roizman, 1990). After packaging of DNA into capsids, the virus matures and acquires infectivity by budding through the inner lamellae of the nuclear membrane. The whole process takes approximately 18–20 h. Enveloped viruses tend to spread from cell to cell leading to progression by local spread. This accounts for the plaques and syncytia of cells seen with virus isolation using cell cultures. Virus may also enter and be transported along the sensory nerves (Section 2.2), to establish latent infections and subsequent disease at other sites.

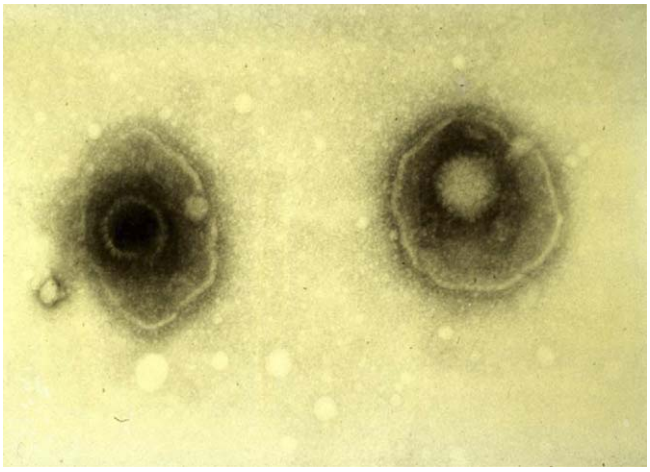


Fig. 1. Electron microscopy of Herpes Simplex virions. Note the central capsid containing the double stranded DNA surrounded by the tegument and envelope.

1.3. Viral proteins

HSV-1 proteins were identified on the basis of proteins contained in virions prior to infection (virion proteins or VP), and proteins accumulating in infected cells (infected-cell proteins or ICP) (Honess and Roizman, 1973; Spear and Roizman, 1972). They have, however, subsequently been named according to their primary function, such as DNA polymerase, or position of the gene along the UL or US segments of the genome (McGeoch et al., 1988, 1991, 1985). The ICP designation, has been retained primarily for five proteins (ICP0, ICP4, ICP22, ICP27, and ICP47) that are the products of immediate–early genes expressed after infection in the absence of de novo viral protein synthesis (Honess and Roizman, 1974, 1975). Synthesis of viral proteins takes place in the cytoplasm with the sequential expression of approximately 80 viral proteins in 3 major kinetic classes: immediate early (IE), early (E), and late (L) genes. The alpha (α) genes and their products the IE proteins are the first to be expressed, reaching peak rates of synthesis 2–4 h post-infection and accumulate until late in infection. All the α proteins have regulatory functions, for example ICP0, 4, 22, 27 are the primary mediators of viral gene expression. The beta (β) genes and their products, the early (E) proteins are dependent on the presence of α gene products. Early proteins reach peak rates of synthesis 5–7 h post-infection and include viral thymidine kinase (TK) and DNA polymerase. A unique characteristic of HSV TK is that its substrate range is far greater than that of its host counterpart, so that it phosphorylates purine pentosides and a wide variety of nucleoside analogues that are not phosphorylated efficiently by cellular kinases. This is the basis for the effectiveness of various nucleoside analogues in the treatment of HSV. The E proteins are involved in viral DNA synthesis and are required for maximum expression of late (L) genes. The products of the late or

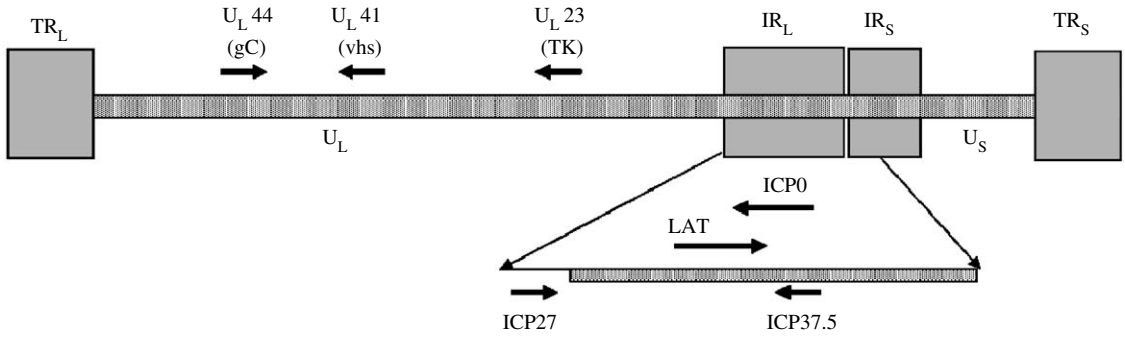


Fig. 2. Linear representation of HSV-1 genome. Terminal long repeat (TR_L), Unique long region (U_L), Intermediate long and short repeats (IR_L and IR_S), Unique short segment (U_S), Terminal short repeat (TR_S). Glycoprotein C (gC), thymidine kinase (TK), UL41 (gene encoding virion host shutoff protein, vhs), latency-associated transcript (LAT), infected cell protein 0 (ICP0). Region coding for LAT overlaps but is on the opposite strand to ICP0.

gamma (γ) genes include virion structural proteins and viral glycoproteins. Gamma (γ) genes are expressed late in infection and those encoding structural proteins are likely to be virulence genes. For example gC and VHS protein-negative mutants have reduced capacity to cause keratitis (Brandt and Grau, 1990; Brandt, 2005) and periocular disease (Smith et al., 2000). Many of the HSV-1 glycoproteins also act to blunt host defences such as blocking the antibody-mediated destruction of the virus.

1.4. Viral genes: functional aspects of the immediate early infected cell proteins

Virulence genes such as those encoding, ICP 0, 4, 22, 27 and 47 play important regulatory roles and are predicted to be virulence determinants for corneal infection because they are required for replication. The gene encoding ICP0, maps in the inverted repeat sequences flanking the unique sequences of UL region (Perry et al., 1986) and has particular significance in the pathogenesis of HSK (Fig. 2). ICP0 is a multifunctional protein (Roizman and Knipe, 2001) and activates transcription from HSV (Cai and Schaffer, 1992; Chen and Silverstein, 1992) either independently or in synergy with ICP4 (Everett, 1986; Quinlan and Knipe, 1985). Newly synthesised ICP0 accumulates near nuclear structures known as ND10 (Maul et al., 1993; Maul and Everett, 1994; Everett and Maul, 1994), the main organising component of which is the promyelocytic leukaemia (PML) protein (Ishov et al., 1999). ND10 structures are a common target of viruses belonging to many diverse families (Everett, 2001; Regad and Chelbi-Alix, 2001). The association of ICP0 with ND10 structures requires ICP4 and ICP27 (Tang et al., 2003). ICP0 promotes degradation and disaggregation of these ND10 structures (Chelbi-Alix and De, 1999; Everett et al., 1998) which is critical for resistance of HSV-1 to interferon. For example, in the presence of interferon, viral gene expression and yields are significantly reduced in PML +ve but not PML −ve cells (Chee et al., 2003). ICP0 together with ICP4, also appears to have an affect on apoptosis by blocking the initiation of the apoptotic response (Boutell and Everett, 2003) (Jerome et al., 2001; Cai et al., 1993). ICP47 may be important in the behaviour of HSV-1 in the cornea through its ability to interfere with the transport of antigens into the endoplasmic reticulum for presentation on the cell surface so that viral antigens are not presented (Hill et al., 1995; York et al., 1994).

1.5. Latency of HSV-1

HSV-1 can establish both productive and latent infections. Infection of non-neuronal cells, usually leads to replication and subsequent death of the host cell. In contrast, when HSV-1 infects sensory neurons, replication is limited, and the virus may become maintained for the lifetime of the host.

1.5.1. Why is neuronal latency so prevalent?

HSV-1 infection results in activation of the host cell's DNA damage repair machinery. DNA repair proteins appear to be beneficial for a productive HSV-1 infection. In neuronal cells, HSV-1 is unable to cause a DNA damage response (Lilley et al., 2005) due in part to the inefficiency of neurons at DNA repair (Ghiasi et al., 2000a, b). In the absence therefore, of a damage response, the integrity of the replication fork or replication intermediates may be compromised, leading to deficiencies in viral replication and contributing to the establishment of latency (Lilley et al., 2005). In contrast in epithelial cell lines, DNA repair proteins are induced in response to a variety of DNA damaging agents or genotoxic stress (Jackson and DeLuca, 2003; Leib et al., 1989b).

1.5.2. HSV-1 genome during latency and lytic infection

The HSV-1 genome has been found to exist in three different states: linear, circular, and concatemeric. In the virion, genomes are linear (Fig. 2). Soon after infection, however, concatemers and branched forms occur. Circularisation from endjoining was thought to occur within hours after infection (Poffenberger and Roizman, 1985), leading to a rolling circle mode of replication (Roizman, 1979; Skaliter et al., 1996). It has more recently been found, however, that that circularisation occurs during the establishment of a latent infection but not during a productive infection (Jackson and DeLuca, 2003). In a productive infection, concatemeric molecules serve as the template for HSV-1 replication (Garber et al., 1993; Jackson and DeLuca, 2003; Sandri-Goldin, 2003), with replication proceeding from 3 origins of HSV DNA replication, to produce highly branched structures. Linear genomes do not persist in cells that are transcriptionally quiet (Jackson and DeLuca, 2003). Viral genomes from the trigeminal ganglion of latently infected animals or quiescent neuronal cells in culture have been found to be maintained in circular forms (Rock and Fraser, 1983; Su et al., 2002). The ICPs are involved in this process, that is, circular genomes persist following an infection with viruses in which all the ICP genes have been deleted (Samaniego et al., 1998). Of the ICPs, ICP0 appears to be intimately involved in this process as well as being a determinant of latency (Jackson and DeLuca, 2003), that is, circularisation of the HSV-1 genome only occurs in cells infected with viruses that do not express ICP0 (Jackson and DeLuca, 2003). The expression of ICP0 which is necessary for efficient reactivation and initiation of infection (Cai et al., 1993; Leib et al., 1989b), may thus regulate the balance between lytic and latent HSV-1 infection (Loiacono et al., 2003). The interaction between ICP0 and nuclear dense (ND) structures (see above Section 1.4) which generally function as sites of DNA double-strand break repair (Carbone et al., 2002), is important for this process. It has been postulated, that the ends of the HSV-1 DNA are treated as double strand breaks leading to joining and resultant circularisation of the genome (Jackson and

DeLuca, 2003; Sandri-Goldin, 2003). The expression, however, of ICP0 would lead to degradation of the cellular proteins in the ND10 bodies and prevent circularisation (Jackson and DeLuca, 2003; Sandri-Goldin, 2003).

1.5.3. Latency-associated transcripts

Virus-specific transcripts have been detected during HSV-1 latency, in the central and peripheral nervous systems of mice and in human trigeminal ganglia (Rock et al., 1987; Stevens et al., 1987). These transcripts, the latency-associated transcripts (LAT), originate from the repeat regions within the long internal and terminal repeats, and are the only abundantly transcribed viral gene during latency (Rock et al., 1987; Stevens et al., 1987). Thus, HSV-1 establishes latent infections as a circular episome associated with histones with active transcription only from the region encoding the LAT. The primary LAT transcript is approximately 8.3 kb long (Dobson et al., 1989; Zwaagstra et al., 1990) and partially or completely overlaps three viral genes, those encoding AL, ICP0, and ICP34.5, in an antisense direction (Rock et al., 1987; Stevens et al., 1987; Perng et al., 2002a) (Fig. 2). A very stable intron, the 2-kb LAT, is spliced from the primary transcript and is the major LAT RNA detected during latency (Farrell et al., 1991; Wechsler et al., 1988, 1989; Zwaagstra et al., 1989).

The LATs although first detectable at the peak of an acute infection, are present at much higher levels during HSV-1 latency with high levels in trigeminal ganglia (Steiner et al., 1988; Stevens et al., 1987). The number of neurons expressing LAT per genome is far higher in the trigeminal ganglion than in the brain stem (Stevens, 1989). In animal models, during an acute infection the transcripts of the HSV-1 genes involved in the lytic cycle increase and then decrease over a 7 day period; while the LATs, first expressed during the acute infection continue to increase thereafter. During reactivation of a latent infection, however, the LATs decrease but remain at significant levels even after reactivated virus is detectable (Spivack and Fraser, 1988). Although the mechanisms involved are not known the establishment and reactivation from latency involves the products of both the ICP0 and LAT genes (Leib et al., 1989b).

While the LAT region has not been shown to encode any proteins, this region has been implicated in a number of pathogenic functions, including neuronal survival (Thompson and Sawtell, 2001), virulence anti-apoptosis (Perng et al., 1999, 2002a; Thompson and Sawtell, 2001), suppression of transcription, establishment of latency (Perng et al., 1999; Thompson and Sawtell, 1997) and reactivation from latency (Leib et al., 1989a). In addition LAT mediates a post-transcriptional constraint on ICP0 protein expression during reactivation (Thompson et al., 2003). There is also evidence that during latency, expression of LAT prevents superinfection of the cell (Mador et al., 2002).

1.5.3.1. LAT and histones. A predominant feature of HSV-1 latency is the general suppression of lytic transcription. This suppression has been shown to correlate with the association of certain modified histones (Kubat et al., 2004). Cellular chromatin is separated into regions that range from permissive to non-permissive for transcription, with pericentric heterochromatin being the most non-permissive and actively transcribed euchromatin being the most transcriptionally permissive (Jenuwein and Allis, 2001). Particular histone modifications lead to chromatin-mediated gene activation (active or euchromatin) or repression (inactive or heterochromatin) (Jenuwein and Allis, 2001). During productive infection, viral DNA is relatively free of nucleosomes (Leinbach and Summers, 1980), and only relatively low levels of viral DNA are associated with the histone H3 (Herrera and Triezenberg, 2004). In contrast, during latent infection, viral DNA is assembled into nucleosomal chromatin (Deshmane and Fraser, 1989; Herrera and Triezenberg, 2004) with the promoter of LAT significantly enriched with the modified histone H3 (Kubat et al., 2004). The latent HSV-1 genome may thus be organised into distinct transcriptional domains, analogous to the organisation of euchromatic and heterochromatic domains in cellular chromosomes. Wang et al (Wang et al., 2005) suggested that HSV-1 uses the *LAT* gene to manipulate cellular histones to assemble heterochromatin in place of euchromatin on viral lytic-gene promoters. Thus LAT region would encompass a transcriptionally permissive domain associated with modified histones typical of euchromatin, while the lytic gene regions would be contained within transcriptionally non-permissive domains, similar to cellular heterochromatin (Kubat et al., 2004).

1.5.3.2. LAT and reactivation. Although HSV-1 mutants with deletions in LAT are able to establish and maintain a latent infection (Hill et al., 1990), LATs, however, appear to be necessary for efficient reactivation from latency (Leib et al., 1989a) although it is possible that this could be explained by LAT's role in increasing numbers of neurons in which latency is established (Thompson and Sawtell, 1997). It has been proposed that LAT may affect the latency reactivation cycle by antisense regulation of the important immediate-early genes for ICP0 and/or ICP4 (Chen et al., 1997; Leib et al., 1989b; Stevens et al., 1987), by association of the LAT with ribosomes (Nicosia et al., 1994), or by expression of a LAT protein that can substitute for an ICP0 function (Thomas et al., 2002). It is the first 1.5 kb of the primary LAT transcript that is important for reactivation (Bloom et al., 1996), a region which contains exon 1 and part of the first intron of LAT. This region of LAT does not, however, overlap with the ICP0 or ICP4 gene and does not contain an open-reading frame (Perng et al., 1996), which suggests that LAT can support reactivation by a mechanism that does require, antisense regulation of ICP0 or ICP4, or expression of a protein (Perng et al., 1996).

1.5.3.3. LAT and apopotosis. Although there has been some controversy regarding LAT's anti-apoptosis activity (Thompson and Sawtell, 2000, 2001), LAT has been shown to have an effect on apoptosis both in vitro and in vivo (Ahmed et al., 2002; Perng et al., 2000) possibly by blocking both major apoptosis pathways (Henderson et al., 2002; Jin et al., 2003).LAT has anti-apoptosis activity in infected tissue culture cells (Henderson et al., 2002; Jin et al., 2004), and LAT-negative mutants appear to have increased apoptosis in rabbit and mouse TG in vivo compared to LAT competent viruses (Ahmed et al., 2002). Although HSV-1 has other anti-apoptosis genes, Us3, ICP27 and glycoproteins Us6 and Us5, LAT is the only anti-apoptosis gene that is expressed at the end of the acute infection, a time when latency is being established when it is crucial to enhance the survival of neurons (Jin et al., 2005). This study, (Jin et al., 2005) also found that restoration of LAT anti-apoptosis ability, led to recovery of reactivation efficacy. This suggests that LAT's anti-apoptosis activity is also important for reactivation (Jin et al., 2003) that is, LAT would prevent apoptosis prior to the completion of reactivation. The authors proposed that decreasing LAT levels allows apoptosis to begin, thus triggering viral reactivation. The restoration of normal LAT levels then attenuates apoptosis, allowing reactivation to go to completion. During latency some neurons are surrounded by T cells, and it has been proposed that these T cells suppress reactivation (Khanna et al., 2003; Liu et al., 2000). Because cytotoxic T cells kill target cells, in part by apoptosis, high levels of LAT in some latently infected neurons may act to prevent the elimination of these neurons by T cells (Jin et al., 2005). The anti-apoptosis function may also serve to increase numbers of neurons that survive during latency establishment, or protect neurons in the maintenance phase of latency increasing the number of neurons available for reactivation.

2. Pathogenesis of HSK

2.1. Entry of HSV-1 into the host and the development of ocular surface disease

HSV-1 spreads usually by direct contact, entering the mucous membrane of the host at the site of contact. Viral replication occurs at the site of inoculation and with spread to other cells, leads to an increase in contact with and entry into sensory nerve endings. The development of HSV-1-related ocular surface disease, principally HSK, represents one or more of 4 events: primary disease, that is, disease occurring in a subject with no previous exposure to HSV-1, initial ocular disease, that is, HSK occurring for the first time in a host who has previously been infected with HSV-1 at another site, recurrent HSK and superinfection with another strain of HSV-1.

The reservoir of HSV-1 is most likely to be the mouth. Indeed, the frequency of asymptomatic salivary compared to tear film shedding (Kaufman et al., 2005; Kaye et al., 1990, 1992) implicates the mouth as the main site for acquisition and spread of HSV-1 in the community.

The development of primary disease depends upon entry of HSV-1 into the ocular surface, where multiplication and local spread to other cells (possibly aided by the tear film) results in clinical disease. Entry of HSV-1 into the cornea has in animal models, required the use of trauma such as scarification (Tullo et al., 1983). Non-traumatic acquisition (Kaye et al., 1992) or inoculation of HSV-1 probably involves entry into the conjunctiva. We have shown that droplet spread to the mouse eye results in the development of a blepharoconjunctivitis similar to that seen in primary HSVO in children (Fig. 3) (Kaye et al., 1992). The development of ocular surface disease from droplet-spread corresponds to both the age of the animal and the strain of HSV-1. Younger animals developed more severe disease, and a greater rise in neutralising antibodies similar to the situation seen in humans (Kaye et al., 1992) (Fig. 4A&B). The development of HSV ocular surface disease from droplet spread was termed the 'front-door' route to the eye (either asymptomatic or symptomatic primary ocular disease) (Kaye et al., 1992; Kaye and Baker, 1996) to distinguish it from the 'back-door' route, that is, spread to the eye from non-ocular site of inoculation (Tullo et al., 1982).

We have shown, in a mouse model, that droplet spread of HSV-1 to the eye using concentrations of virus found in saliva, results in direct entry into the ocular surface with

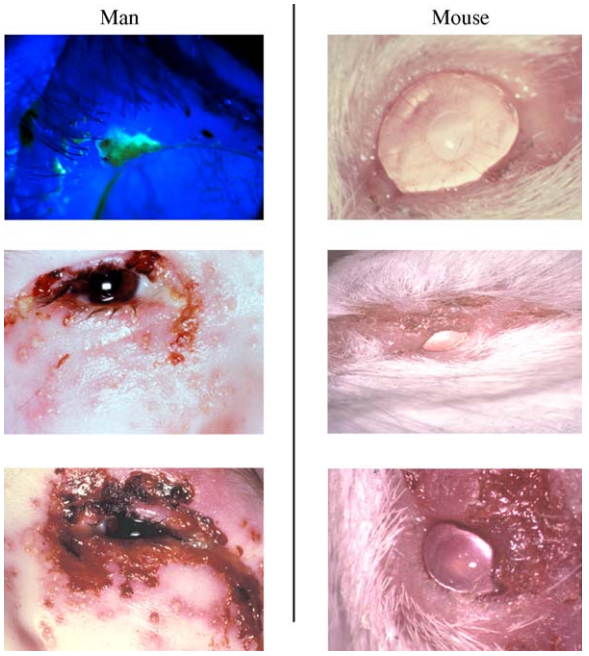
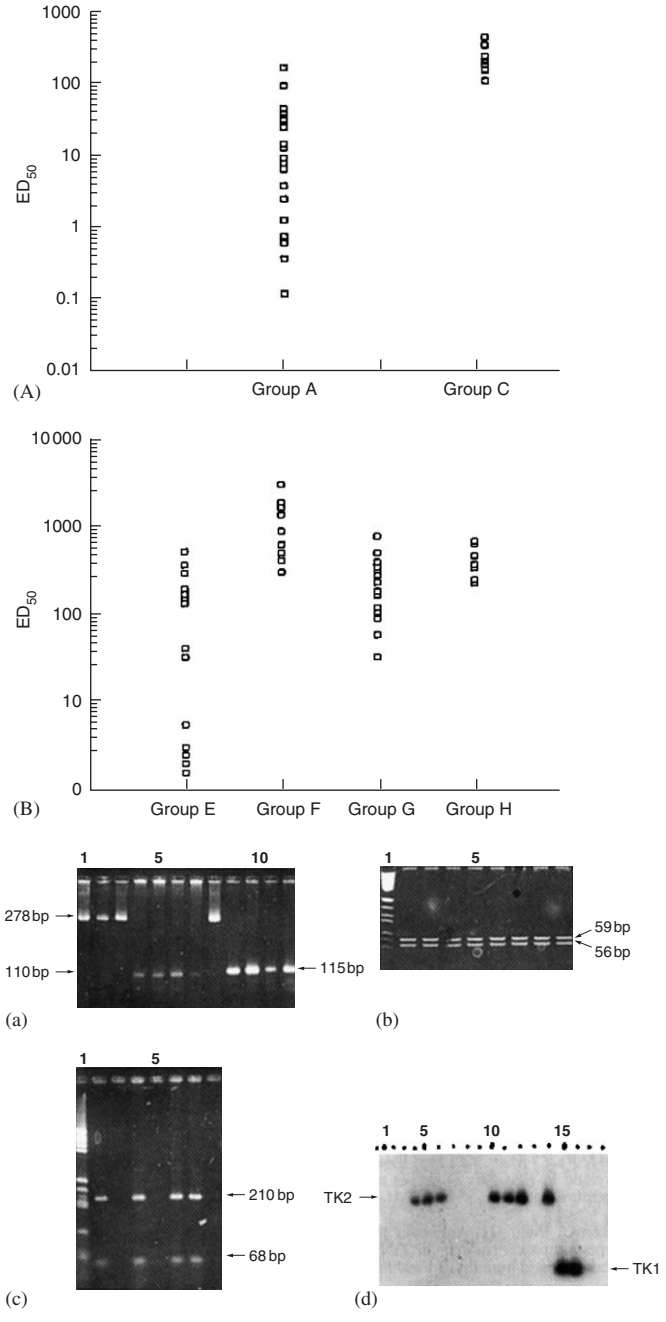


Fig. 3. Droplet spread of HSV-1. Development of blepharitis and conjunctivitis following droplet spread of HSV-1 on the mouse eye. Note the similarity of the ocular surface disease (blepharitis and conjunctivitis) in the mouse and human eye. Younger mice developed more severe disease and a greater neutralising antibody reponse. (Reprinted with permission from Kaye, S.B., Shiemld, C., Grinfeld, E., Maitland, N.J., Hill, T.J., Easty, D.L. (1992) Non-traumatic acquisition of HSV infection through the eye. BJO 76, 412–418.)

HSV-1 reaching and becoming latent in the cornea, iris and trigeminal ganglion (Kaye et al., 1992). This may lead to subsequent recurrence in the cornea. In the murine model, a significant proportion of cases using droplet spread have little clinical disease, but latency is still established in the trigeminal ganglion and cornea (Kaufman et al., 2005; Kaye et al., 1992). A parallel can be drawn with the incidence of asymptomatic primary ocular disease where it is estimated that primary infections manifest clinically in only 1-6% of occasions (Umene and Sakaoka, 1999). Asymptomatic primary herpetic eye disease may thus play an important role in the development of recurrent corneal disease.



Initial ocular disease may also occur following acquisition of HSV at a non-ocular site and subsequent spread to the eye. Inoculation of HSV-1 in the lower lip or snout of mice results in HSV-1 becoming latent in the all three divisions of the trigeminal ganglion. This may then potentially allow virus to reach the eye (Tullo et al., 1982). Until fairly recently, it was thought that the development of recurrent disease, always involved reactivation of HSV-1 in an already infected host. It has, however, been shown that infection by a different strain of HSV-1 may occur in a previously infected host. This has been shown for both genital herpes as well as ocular HSK (Remeijer et al., 2001, 2002).

2.2. Transport of HSV-1 to and from the cornea

Whether HSV-1 enters via the eye or the mouth, virus has been found and recovered from the trigeminal ganglion, brainstem and cornea. Virus is transported in neuronal axons toward nerve cell bodies, and latency is established in sensory ganglia.

The nucleocapsid of the virus moves by retrograde transport within the peripheral axons to the nuclear compartment of the sensory cell. Upon replication in the neuronal cell body, new components of the virion move by anterograde transport from the neuron cell body to both peripheral and central branches of the neuron (Lavail et al.,

Fig. 4. (A) Neutralising antibody response expressed as the ED50. Group A, topically inoculated with 5×10^5 pfu HSV-1 strain McKrae; group C, scarified with 5×10^3 pfu HSV-1 strain McKrae. Groups E and G, topical inoculation with 10^6 pfu HSV-1 strain SCI6 or McKrae, respectively. Groups F and H, scarified with 10^4 pfu HSV-l strain SCI6 or McKrae, respectively. Note the greater antibody response in the topical groups with McKrae compared to SC16. Both disease severity and neutralising antibody response was greater in younger mice. (Reprinted with permission from Kaye, S.B., Shiemld, C., Grinfeld, E., Maitland, N.J., Hill, T.J., Easty, D.L. (1992) Non-traumatic acquisition of HSV infection through the eye. BJO 76, 412–418). (B) Polymerase chain reaction. (a) 3% agarose gel. TK2 amplification product (278 bp): lanes 1–3 (cornea, iris, and trigeminal ganglion (TG), topical inoculation with 5×10^5 pfu HSV-1 McKrae), lane 8 (TG scarified with 5×10^3 pfu HSV-1 McKrae); TK1 amplification product (110 bp): lanes 4–6 cornea, iris and TG, scarified 5×10^3 pfu HSV-1 McKrae), lane 7 (cornea, topical inoculation 5×10^5 pfu HSV-l McKrae), gC amplification product (115 bp): lanes 9 and 10 (cornea and TG scarified 5×10^3 pfu HSV-l McKrae), 11 and 12 (cornea and TG, topical inoculation 5x 105 pfu HSV-1 McKrae). (b) 12% polyacrylamide gel. Lanes 1–8, Ksp I digest of gC amplification product (59 and 65 bp), lane 9 (Hae111 digest of pBR322 plasmid DNA). (c) 3% agarose gel, Sma 1 digest of TK2 product (210 and 68 bp). Lane l, marker (Hae111 digest of pBR322 plasmid DNA). (d) Autoradiograph 3 h: 5′ end labelled probe from a 26 base oligonucleotide for TKI (110 bp) and TK2 (278 bp) amplification products. Lanes 1–3 (TG, uninfected and mock infected), lanes 4–6 (cornea, iris and TG, 5×10^5 pfu HSV-1 McKrae topical infection), lanes 7–9 and 13 (gC amplification products), lanes 10–12 (cornea, iris and TG, scarified with 5×10^3 pfu HSV-l DNA McKrae), lane 14 (HSV-l McKrae), lane 15 (TG scarified with 5×10^3 pfu HSV-1 McKrae), lane 16 (HSV-1 McKrae), lanes 17 and 18 (TG, mock infected and uninfected). (Reprinted with permission from Kaye, S.B., Shiemld, C., Grinfeld, E., Maitland, N.J., Hill, T.J., Easty, D.L. (1992) Non-traumatic acquisition of HSV infection through the eye. BJO 76, 412–418.)

2005). Little, however, is known about the exact mechanisms of viral DNA transport or release from the axon. Movement of HSV-1 from the nerve cell bodies of infected neurons to the periphery may involve transport by means of microtubule motors (Penfold et al., 1994; Tomishima et al., 2001; Tomishima and Enquist, 2001). There is evidence that nucleocapsids and viral membrane glycoproteins move toward axon termini on different sets of axonal microtubules, with assembly of envelopes onto capsids at axon termini (Tomishima and Enquist, 2001). The mechanism that allows encapsulated viral DNA to be sorted to the axon compartment is not known. It has been assumed that viral DNA is released along the axon shaft and terminal, because the glial cells, which envelop the infected axons, also become infected with HSV (Ohara et al., 2001). It is not clear whether transcytosis or transneuronal transfer occurs by the replication in each cell body in a synaptic chain. Lavail et al. (2005) demonstrated that viral-DNA replication must occur for HSV-1 to move to and be transported anterograde down the length of the axon in detectable amounts at a rate of about 0.5 mm/h. Virus that is incapable of replication, fails to be delivered to the axon compartment (Lavail et al., 2005). It is important therefore, for HSV-1 to multiply in the ganglion before the immune system has responded. Within the trigeminal ganglion, infection is largely restricted to neurons with little intraganglionic spread. It is probable that control of multiplication by CD8+ cytotoxic lymphocytes decreases potential spread into the brainstem.

It is believed that cycles of HSV reactivation in latently infected neurons, accompanied by anterograde axonal spread to the cornea, lead to recurrent infections and scarring of the cornea (Shimeld et al., 1989, 1990). HSV-1 reactivation may be triggered by stress such as UV light and surgery (Dhaliwal et al., 2001; Shimeld et al., 1989). This is, however, not exclusive and recurrent ocular disease does not entirely depend on anterograde transport with disease recurring from local (corneal) reactivation.

In the murine cornea, HSV-1 predominantly replicates and spreads in the epithelial layers of the cornea. Throughout this infection there are very low or undetectable levels of viral proteins in the stromal layer, which is separated from the epithelium by a basement membrane that likely impedes virus movement into the stroma (Polcicova et al., 2005). Corneal stromal disease, however, begins after most viral antigens are cleared from the epithelium. Although it has previously been suggested that stromal keratitis requires HSV-1 transport to sensory ganglia followed by return to the corneal stroma (Streilein et al., 1997), there is substantial evidence that HSV-1 can persist, either as infectious virus or in a latent state, for long periods within the cornea. In addition, viral DNA and infectivity are frequently found in human tears and in corneas removed for transplantation (Fukuda et al., 2003; Neufeld et al., 1999; Openshaw et al., 1995; Remeijer et al., 2001; Kaye et al., 1992, 2000). Corneas may also be damaged by reactivated virus during storage (Remeijer et al., 2001; Robert et al., 2003; Biswas et al., 2000). In addition, in rabbits and mice, persistent HSV-1 DNA has also been detected in the corneas long after acute infections have subsided (Sabbaga et al., 1988; Robert et al., 2003; Mitchell et al., 1994). Therefore, the presence of persistent HSV-1 in the cornea may provide an alternative source of viral antigens that trigger HSK. Thus, rather than taking a roundtrip to the nervous system, HSV-1 resident in the cornea may cause HSK. Polcicova et al. (2005) constructed an HSV mutant that replicated normally in the cornea but was unable to return to the cornea from sensory ganglia. The HSV protein, US9, functions to promote movement of viral membrane glycoproteins to axon termini and in the absence of US9, no infectious progeny are produced at axon tips, that is, it could not travel in an anterograde direction (Polcicova et al., 2005). As with anterograde transport, retrograde transport from axon termini to nerve cell bodies involves axonal microtubule motors that carry capsids to the nucleus, where virus replication occurs. HSV-1 without US9, however, was able to move from the cornea to the ganglia normally and was not required for retrograde spread to sensory ganglia. Thus, HSV-1 lacking US9 can travel in a retrograde, but not anterograde direction (Polcicova et al., 2005) and importantly virus that had previously been introduced in the cornea was able to cause recurrent HSK.

This provides evidence that HSK may also result from virus which has become latent or persists in an infectious state within the cornea (Polcicova et al., 2005). Although, it is possible that HSV could potentially leave the cornea with trafficking immune cells and then return at a later time with these cells, there are numerous observations of HSV either persistent or latent in human corneas (Fukuda et al., 2003; Neufeld et al., 1999; Openshaw et al., 1995; Kaye et al., 1992, 2000). As discussed below, this latter situation is much more likely to account for herpetic stromal disease. Furthermore, the observations that anterograde spread from ganglia to cornea is not required for disease, implies that HSV-1 that remains in the cornea can cause HSK (Polcicova et al., 2005). Although, HSV-1 could conceivably come with immune cells through the epithelium (see Section 2.5) or in the new blood vessels with blood-ocular barrier breakdown, it is apparent that HSV-1 resident in the cornea can initiate stromal disease.

2.3. Non-neuronal sites of sites of latency: HSV-1 in the cornea

Although, the trigeminal ganglion is the primary site for harbouring latent HSV-1 in terms of eye disease, there is considerable evidence that the cornea also harbours latent virus. HSV-1 was isolated following prolonged culture of the corneas of patients undergoing penetrating keratoplasty who had no clinical evidence of active herpetic disease raising the possibility of HSV-1 latency within the cornea (Easty et al., 1987). The detection of viral antigens in the cornea (Holbach et al., 1990, 1991) was more

difficult to interpret. Other studies followed, which confirmed the recovery of HSV-1 from the cornea following prolonged organ culture in between 2% and 25% of cases (Cook et al., 1986, 1991; Kaye et al., 1991, 2000). Because of the difficulty in differentiating latent virus from a low-grade infection, molecular techniques—PCR and in situ hybridisation (ISH) were subsequently used in an attempt to answer this question. There followed several studies showing the presence of HSV-1 DNA in human corneas (Kaye et al., 1991; Cantin et al., 1991; Crouse et al., 1990).

Abghari et al. (1992) demonstrated LAT in the mouse cornea using ISH on dissociated corneal cells. In contrast, however, Romanowski et al, also using ISH, were unable to detect LATs in the corneas of latently infected mice and rabbits. The detection of LAT using PCR has, however, been more informative. Another study (Cook et al., 1991) found expression of LAT in two out of nine rabbit corneas 41 days after inoculation. In contrast, O'Brien et al. (1998) found LAT expression only in acutely infected but not latently infected rabbit corneas. It should be mentioned that there is spontaneous reactivation in the rabbit model. Kaye et al. (1992), using RNA-PCR, found evidence of LAT in eight human HSK corneas, in the absence of gC transcription. In contrast, however, in a study of 18 human patients with a history of HSK that was inactive at the time of surgery, and that had been treated with antivirals but not steroids, neither LAT nor transcripts coding for alpha proteins were detected by ISH (Rong et al., 1991). The difference between the results for PCR and ISH may be due to the difference in their relative sensitivities, particularly if there is very low-level expression and sampling error as well as the duration of existence of HSV-1 in the cornea. The widespread use of topical antivirals, particularly aciclovir is also likely to affect amount of HSV-1 RNA expressed.

Although, there are no studies directly comparing the duration of existence of HSV-1 in the trigeminal ganglion and cornea, the available evidence suggests that detection in the cornea may be time dependent. In a study evaluating the detection of HSV-1 using IHC, ISH, culture and PCR on the corneas of 110 patients undergoing penetrating keratoplasty for herpetic (52 patients) and non-herpetic (58 patients) disease, HSV-1 DNA was detected significantly more frequently in the corneas of those patients who had their last documented episode of HSK within 2 years of surgery (Kaye et al., 2000). In particular, patients who had less than 50 genome equivalents of HSV-1 DNA, in their corneas, had a median and mean time of 1.75 and 1.80 years post-surgery compared to 0.75 and 0.65 years for those patients with more than 50 genome equivalents (Kaye et al., 2000). As a corollary, patients with HSK but no evidence of HSV-1 DNA had median and mean times of 5.00 and 8.56 years. Similar results were obtained for the presence of HSV-1 antigen. The presence of HSV-1 antigen would indicate more recent HSV-1 replication possibly from local reactivation, in contrast with the finding of LAT in absence of other transcripts. These results suggest that HSV-1 has a time-dependent existence in the cornea and possibly reflects the fact that the cells containing HSV-1 in the cornea are more metabolically active than those in the trigeminal ganglion. HSV-1 antigen was also detected significantly more frequently in the stroma rather than the epithelium or endothelium (Kaye et al., 2000). Whether this reflects a barrier effect of Descemet's membrane and the greater turnover and or post-mitotic state of the surface cells of the epithelium is unclear, but points to the stroma as the site for latency or persistence of HSV-1. These results are also of practical significance in terms of the management and treatment of HSV-1 keratitis (Section 6). Although, it is possible that the presence of HSV-1 DNA may represent the persistence of defective viral genomes there is little evidence to support this. Rong et al., (1991) found that both the TK and LAT regions of genes were intact in 72% (13/18) of sampled human corneas and evidence from our laboratory found that regions coding for the major capsid protein, thymidine kinase and glycoprotein C were all found to be consistently present in the cornea suggesting intact viral genomes (Kaye et al., 1991, 1992).

Taken together, the recovery of HSV-1 following prolonged culture, the consistent detection of HSV-1 DNA with evidence suggestive of intact viral genomes, the possible presence of LAT and the ability of corneal cells to regulate the LAT promoter in a similar manner to neuronal cells (Perng et al., 2000) provides strong evidence for the ability of HSV-1 to establish a latent or persistent infection in the cornea. This is supported by the work of Polcicova et al. (2005) which showed that reactivation in sensory neurons is not required for recurrent HSK.

2.4. Superinfection

Recurrences occur despite acquired immunity. There is good evidence that superinfection with a different strain of HSV is possible. This has been noted for both genital and ocular disease. Both recurrent genital and ocular herpetic disease has been found to be due to reactivation or reinfection with a new strain. Remeijer et al found that a third of corneas with recurrent disease were superinfected with a different HSV-1 strain and 63% with the same strain in recurrent HSK (Remeijer et al., 2001, 2002). One patient in their study with bilateral HSK had different strains in each cornea. It would thus appear that recurrent disease might be due to either reactivation or reinfection with a new strain. Although it would appear that superinfection occurs in the cornea, it is not clear whether this translates to superinfection within the trigeminal ganglion. Expression of LAT appears to interfere with superinfection by other HSV-1 strains (Mador et al., 2002) and latency in the trigeminal ganglion is accompanied by a chronic immune response, which may protect from superinfection (Theil et al., 2003).

2.5. The immune response

Corneal, conjunctival or lid epithelial disease is initiated by infectious virus that replicates in the epithelial cells and studies in animal models have shown the requirement of viral replication for this phase (Babu et al., 1996). A threshold effect is observed for the inoculum size; in that once infection is initiated it proceeds to completion unless viral load is reduced (Kintner and Brandt, 1995). Corneal epithelial disease induces an influx of innate immune cells such as polymorphonuclear leucocytes (PMN), macrophages, natural killer (NK) cells and other mononuclear cells into the underlying stroma. PMNs in response to chemokines, escape from limbal vessels and dominate the early inflammatory phase peaking at 48 h (Thomas et al., 1997) and contribute to virus removal (Thomas et al., 1997; Tumpey et al., 1996). Different explanations have been offered for the mechanism of virus removal, which include production of TNFα, nitric oxide and reactive oxygen metabolites by PMN (Daheshia et al., 1998). In addition to ICP0 inducing chemokine production (Thomas et al., 1998), HSV-1 DNA may have Toll-like receptor (TLR-9 and TLR-2) binding activity leading to the synthesis and secretion of cytokines (Lund et al., 2003; Kurt-Jones et al., 2004). Pro-inflammatory cytokines such as interleukin (IL)-1α, IL-1β, IL-8, IL-6, IL-12, IL-17, interferon (IFN)-α, tumour necrosis factor (TNF)-α, macrophage inflammatory protein (MIP)-2, monocyte chemotactic proteins (MCP)-1, IL-12 and MIP1-α are also released by the infected and neighbouring cells, PMN and other cells in the early response (Stumpf et al., 2001; Brandt, 2005). PMN are also a source of vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP)-9 which form a crucial role in the development of corneal vascularisation (Zheng et al., 2001a; Lee et al., 2002b) (Section 2.6). A transient corneal clouding may also occur due to the toxic effect of cytokines on the endothelial cells. Langerhans cells, likely released from the conjunctiva in response to the infection, may present the HSV-1 antigens to T cells in draining lymph nodes (Jager et al., 1992). The role of NK cells and macrophages is ill-defined in HSK and there is now a suggestion that they may participate in virus clearance and also act as a source of cytokines, chemokines and angiogenic factors. Depletion of NK cells and macrophages following HSV-1 infection accelerates HSK and macrophages from the regional lymph nodes are the key players in the process (Bauer et al., 2000, 2001; Tamesis et al., 1994).

This association of long-term persistence of HSV-1 in ocular tissues and chronic inflammation has been demonstrated in several animal studies (Babu et al., 1996; Maggs et al., 1998; Mitchell et al., 1994). The likelihood of HSV-1 persistence either as a low-grade infection or following repeated reactivation from latency or entry into the cornea from sensory nerves would for example, justify the need to use antivirals when treating all forms of herpetic keratitis. The importance, however, of the immune response in stromal keratitis is apparent from the observations that in T-cell-deficient mice, ocular infection with HSV-1 was not associated with the development of marked stromal disease (Metcalf and Kaufman, 1976; Streilein et al., 1997). Immune response to HSV-1 infection can include T-cell-mediated delayed-type hypersensitivity that may be critical to elimination of the virus. Viral spread may be prevented by cytotoxic T lymphocytes and neutralising IgG antibodies. CD4+ Th1 cells are the immune cells most commonly implicated, which mediate inflammation through secretion of IL-2, INFγ and TNFα. CD8+ cytotoxic T lymphocytes have, however, also been linked to an increased incidence and severity of HSK. Although CD4+ Th2 cells, which secrete IL-4, IL-5, IL-6 and IL-10 and have been shown to produce HSK in animal models, their role is less clear. HSK can also result from an antibody dependent, complement-mediated inflammatory insult. NK cells display surface receptors for the Fc component of IgG and may mediate antibody-dependent cell-mediated toxicity. Further support for this mechanism comes from observations that immunisation of mice with glycoprotein K results in more severe keratitis upon subsequent infection (Brandt, 2005; Streilein et al., 1997). Humoral immunity has been shown to have a protective effect if present in sufficient concentrations immediately after infection (Shimeld et al., 1990) and its suppression can reduce the severity of HSK (Jordan et al., 1983).

HSV-1-specific T lymphocytes may also persist in corneas and memory T and B lymphocytes specific for T and B lymphocytes circulate in the blood and lymph armed for the next encounter with the virus. There is some evidence that a chronic low-level viral infection persists in the stromal keratocytes providing a continuous stimulus to antigen-specific T lymphocytes that kill the keratocytes by a cytotoxic mechanism (Carr et al., 2001). Several hypotheses exist for the agents, which drive the CD4+ T cells to orchestrate the inflammatory process. For example, there is a role for the bystander activation of T cells, dependent on continuous viral replication with release of cytokines (Deshpande et al., 2001). It was suggested that an epitope encoded by the UL6 gene of HSV-1 can mimic a corneal antigen ($IgG2a^b$ Ig isotype) and initiate an immune attack on corneal antigens (Avery et al., 1995; Maggs et al., 1998; Zhao et al., 1998), although there is now evidence against this. Ellison et al., analysed the UL6 gene sequence of ocular viral isolates from patients with varying patterns of recurrent HSK but found no polymorphisms that would predict altered expression of the UL6 (299–314) epitope (Ellison et al., 2003). It is also possible that antigen expression may result from host transcription of viral DNA and class I MHC presentation of the viral protein (Fynan et al., 1995; Ulmer et al., 1993). The presence, however, of an active inflammatory infiltrate in the corneas of patients with HSK, together with the finding of persistent HSV-1 DNA and antigen (Tullo et al., 1985), makes either a low-grade productive infection or low-grade reactivation from a latent virus a distinct possibility.

Replication of HSV-1, however, is required to produce disease mediated through recognition of viral antigens by T lymphocytes (Streilein et al., 1997) although a T-cell response is present in the trigeminal ganglion during latency (Theil et al., 2003). If, however, HSK is due to an immune response to an HSV-1 antigen, ICP0 is a likely candidate (Naito et al., 2005). If latency of HSV-1 in the cornea is similar to latency in the trigeminal ganglion, then production of ICP0 would require reactivation of virus. Possible sites of HSV-1 reactivation are either locally within the cornea or peripherally within the trigeminal ganglion.

As discussed above (Section 2.3) there is substantial evidence that HSV-1 may remain in the cornea, either in a latent or low-grade persistent infection. It has been unclear, however, whether recurrent HSK is due to reactivation of virus within the cornea or in the trigeminal ganglion with anterograde transport to the cornea. The time interval required to produce recurrent HSK following inoculation of HSV-1 into the cornea and subsequent UV irradiation, is suggestive of the round-trip hypothesis but does not exclude local that is, corneal reactivation. HSV-1 can cause HSK in mice without a roundtrip to the nervous system (Polcicova et al., 2005) indicating that recurrent HSK may be due to local reactivation within the cornea rather than reactivation in the trigeminal ganglion. It is possible therefore, that in human beings, HSV-1 may not only return to the cornea from the trigeminal ganglion, but might also lead to HSK from virus, which has become latent or persistent within the cornea. Furthermore, the observation that anterograde spread from ganglia to cornea is not required for disease, contradicts the notion that small amounts of HSV antigens arriving in the stroma from infected neurons can promote HSK (Polcicova et al., 2005). Although it has been suggested, that professional antigen-presenting cells, such as Langerhans cells or monocyte–macrophages, acquire viral antigens in the epithelium, before promoting inflammation in the stroma (Polcicova et al., 2005) the significantly greater prevalence of HSV-1 antigen in the stroma compared to the epithelium or endothelium (Kaye et al., 2000) points to the stroma as the site to which the immune response is directed.

2.6. Corneal scarring and vascularisation

The pathogenesis of corneal scarring and vascularisation in HSK is still uncertain. There are conflicting reports in the literature as to the protective effects of CD4+, CD8+ or both in corneal scarring (Ghiasi et al., 2000a). It has also been suggested that Th1 (IL-2) responses may protect and Th2 (IL-4) responses may enhance scarring (Ghiasi et al., 1999). Similar controversies exist on the role of NK cells and macrophages (Ghiasi et al., 2000b). One of the major events after HSV-1 infection is the production of proinflammatory cytokines and chemokines and an invasion of the cornea by PMN. This response helps in clearing the virus but at the same time lends entry to various cytokines and angiogenic factors secreted by the PMN. IL-1 and 6 are important mediators of inflammation and a beneficial effect of IL-ra (interlukin-1 receptor antagonist) has been noted on disease severity and corneal scarring (Biswas et al., 2004a). IL-10 and 12 can also suppress HSK lesions in animal models possibly by induction of antiviral cytokines such as interferon γ, cellular defences like NK cells and T-cell apoptosis. IL-12 also has an antiangiogenic effect (Lee et al., 2002a). Co-ordinated phenotypic changes, extracellular matrix (ECM) deposition and remodelling are the key elements in the process of tissue repair as in corneal scarring. Various cytokines and growth factors are involved and the most important of these are epidermal growth factor (EGF) and transforming growth factor β (TGFβ) (Schultz et al., 1992). TGFβ up-regulates fibroblast proliferation and ECM synthesis and reduces matrix degradation after injury. One of the major challenges has been in understanding the differences between foetal (scar free) and adult wound healing (scarring). It is now known that "matricellular proteins", a group of disparate proteins expressed during development but not in adults are up-regulated in sites of tissue re-modelling and act temporally and spatially to provide regulatory signals in cell–cell and cell–matrix interactions (Bornstein and Sage, 2002). One of the matricellular proteins, extensively studied in the corneal in vivo models, is the platelet-derived glycoprotein thrombospondin (TSP). Thrombospondins are a family of five glycoproteins the first two of which TSP 1 and TSP 2 are involved in wound healing and are potent anti-angiogenic agents (Bornstein et al., 2004; Armstrong and Bornstein, 2003). Given that TSP 1 and 2 play an important role in corneal scarring and vascularisation the next question is their source in the cornea. One mechanism could be by invading blood vessels in the cornea, which have been shown to appear as early as 24 h after infection in vivo (Lee et al., 2002b). Scarring, however, also develops in an avascular cornea, where fibroblasts and not platelets are the predominant cells. This led to the search for a local reservoir of TSP in the cornea. Work from our lab (Fig. 5) has shown this reservoir to be keratocytes, which express TSP 1 and 2 in an in vitro stromal wound repair model (Hiscott et al., 1999; Choudhary et al., 2005). TSP 1 acts by modulating cellular responses to ECM and can also bind and activate TGFβ (Bornstein et al., 2004). Corneal vascularisation in HSK requires active viral replication and procedures that minimise angiogenesis may diminish the severity of HSK (Zheng et al., 2001a, b; Lee et al., 2002b). Angigogenesis has been demonstrated as early as 24 h post-infection, thereby supporting the role of corneal vascularisation in the severity of HSK (Zheng et al., 2001b). HSV-1 differs from other viruses in that it does not encode molecular mimics of any known angiogenic factors. This suggests that HSV-1 infection may disrupt the normal equilibrium between angiogenic and anti-angiogenic stimuli leading to an "angiogenic switch" initiating angiogenesis (Sottile, 2004). HSV-1 infection can induce the production of many angiogenic factors such as VEGF,

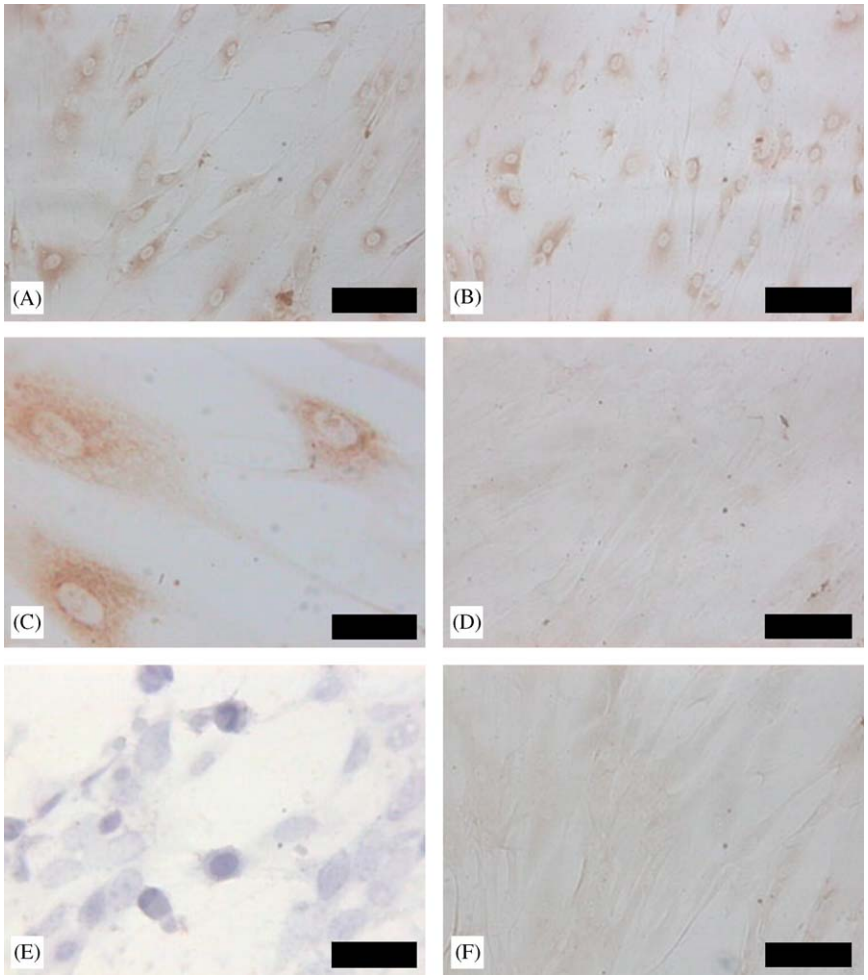


Fig. 5. Immunohistochemical staining of keratocytes for TSP 1 and TSP2. (A, B) Non-confluent cultured keratocytes labeled for TSP1 (A) and TSP2 (B) (DAB staining). (C) TSP1 immunoreactivity was localised in a diffuse granular perinuclear pattern with peripheral punctuate staining. (D) No staining could be visualised in the control procedures in which primary antibody was replaced by IgG fragments. (E) Keratocytes have a rounded configuration at 72 h after infection with HSV1 (haematoxylin & eosin). (F) A clear reduction in the protein signal (compared to B) is seen for TSP2 at 4 h. (DAB staining). The scale bars represent 54 μm in (A, B, D, F) and 50 μm in (C, E). (Reprinted with permission from Choudhary, A., Hiscott, P., Hart, C. A., Kaye, S. B., Batterbury, M., and Grierson, I. (2005) Suppression of thrombospondin 1 and 2 production by herpes simplex virus 1 infection in cultured keratocytes. Mol. Vis. 11, 163–168.)

MMP 2 and 9, platelet-derived growth factor (PDGF), beta fibrosing growth factor (bFGF), MIP-2 and MCP-1 (Zheng et al., 2001a; Yang et al., 2003; Lee et al., 2002b). Hypoxia due to corneal oedema may also serve as an angiogenic stimulus. The source of these factors seems to be mainly PMN but VEGF is also expressed in epithelial, endothelial and stromal cells. The angiogenesis cascade likely involves cytokine mediated and other paracrine effects as discussed above. IL-1 and IL-6 are potent stimulators of VEGF production and IL-1 receptor antagonist results in reduced angiogenesis in HSV-1 infection (Biswas et al., 2004b). VEGF expression appears to be a paracrine effect as the cells expressing the protein do not necessarily express HSV-1 antigen (Zheng et al., 2001a). But, what contributes to these changes? IL-6 produced from virus infected cells can stimulate non-infected resident cells and other inflammatory cells in a paracrine manner to secrete VEGF (Biswas et al., 2006). It has been shown that the genome of HSV, like the genome of bacteria but unlike the human genome contains a number of CpG motifs (Zheng et al., 2002). CpG motifs have been shown to stimulate multiple cellular components of the immune and central nervous system (Klinman et al., 2000). Zheng et al. (2002) were able to demonstrate that potentially bioactive CpG motif in HSV DNA and/or its breakdown products contribute to corneal angiogenesis by inducing the production of VEGF. Further, small interfering RNA (siRNA) targeting of the VEGF pathway can inhibit ocular angiogenesis induced by bioactive CpG and HSV infection and may represent a useful therapy for HSK (Kim et al., 2004). These angiogenic responses are balanced by anti-angiogenic influences which include cytokines such as IL-12 and IL-18, TGF β, tissue inhibitors of metalloproteinase (TIMP) and TSP 1 and 2 (Yang et al., 2003; Armstrong and Bornstein, 2003). IL-12 and 18 are up-regulated following HSV-1 infection and inhibit

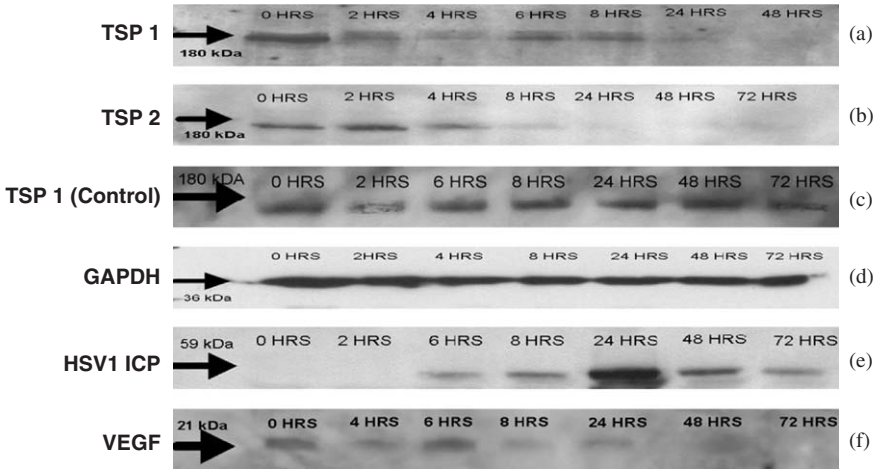


Fig. 6. Western blot analysis. Protein expressions of TSP 1, TSP 2, TSP 1 (control), GAPDH, HSV1:ICP27 and VEGF at different time intervals post-infection. Densitometric analysis revealed a 50% reduction in the signal intensity of TSP 1 and 2 by 8 h, with a complete absence by 24 h post-infection (a, b). There was no change in the expression of TSP 1 in sham-infected cultures (c). There was no change in the expression of GAPDH at all times post-infection (d). Immediate early viral proteins (HSV-1:ICP27) were expressed by 6 h, reaching maximum intensity at 24 h post-infection (e). VEGF expression continued till after TSP 1 and 2 expressions had ceased (f). (Reprinted with permission from Choudhary, A., Hiscott, P., Hart, C. A., Kaye, S. B., Batterbury, M., and Grierson, I. (2005) Suppression of thrombospondin 1 and 2 production by herpes simplex virus 1 infection in cultured keratocytes. Mol. Vis. 11, 163–168.)

angiogenesis by down-regulating VEGF expression. In a recent study, Kim et al. (2005) have shown that IL-18, an endogenous negative regulator of HSV-1-induced angiogenesis results in reduced stromal keratitis lesion severity. While some chemokines, such as MIP-2 are angiogenic others such as CXC chemokines lacking the E–L–R motifs may have an angiostatic effect, and may function by binding to heparin sulphate on target cells. As heparin sulphate is also involved in HSV-1 entry into target cells it is likely that HSV may bind heparin sulphate thus blocking the angiostatic activity and driving the process towards angiogenesis (Cao et al., 1995; WuDunn and Spear, 1989; Luster et al., 1995). As discussed earlier TSP 1 and 2 are potent anti-angiogenic agents expressed by keratocytes and can also bind and inhibit the activity of bFGF, VEGF, MMP2 and MMP9 (Armstrong and Bornstein, 2003). HSV-1 infection can selectively suppress matrix protein synthesis and we have shown (Figs. 5 and 6) that TSP 1 and 2 are selectively down regulated by infection in human keratocytes in vitro (Choudhary et al., 2005). We also noted that keratocytes expressed VEGF after infection and its production continued till after TSP 1 and 2 had ceased (Choudhary et al., unpublished findings). These findings support the hypothesis of alteration of the normal balance between angiogenic and anti-angiogenic responses as the likely cause of corneal vascularisation in HSK and may form the basis for the next generation of treatment options for this condition.

3. Outcome of infection

The pathogenesis of HSV-1 disease is dependent on a number of factors, including the viral genes encoded by each HSV-1 strain and the genetic make up of the host and host immune system.

3.1. HSV-1 strains

A strain refers to a single viral isolate obtained from an infected individual. Several hypervariable regions have been identified in the HSV-1 genome, which encompass unique tandemly repeated sequences, or reiterations that vary in copy number and nucleotide sequence (Rixon et al., 1984). HSV-1 strains can be differentiated by analysing these variations or restriction fragment length polymorphisms, which can be due to a gain or loss of a retriction endonuclease cleavage site (Buchman et al., 1978) or variations in the length of cleaved fragments (Umene, 1998). Different HSV-1 isolates have been associated with different severities of corneal disease, ranging from relatively asymptomatic to severe disease and corneal scarring (Brandt and Grau, 1990). Several viral genes in combination with host factors influence the virulence of the strain. For example, ICP34.5, thymidine kinase, ribonucleotide reductase and the US3 protein kinase genes have been linked to neurovirulence. The McKrae strain, is a prototypic virulent HSV-1 strain, while in contrast the KOS strain is much less virulent and differs in the number of Pro–Ala–Thr repeats in the ICP34.5 gene product (Mao and Rosenthal, 2003; Perng et al., 2002b). The initial infecting viral strain usually colonises the sensory ganglia and is usually thought to be the cause of recrudescent infections (Asbell et al., 1984) although superinfection with a new strain is also possible (Remeijer et al., 2002). Overall mutation rates for HSV-1 have been estimated to be 3.5×10^{-8} mutations/site/year (Umene, 1998). This is less than that, described for RNA viruses and does not explain

intra-individual HSV-1 genotype differences (Umene, 1998). This suggests a possible role of super-infection with other strains and generation of recombinant viruses, possible with enhanced virulence. Norberg et al. (2004) demonstrated both intra and intergenic recombinants and suggested that most full-length HSV-1 genomes consist of a mosaic of segments from different genetic groups. The link between genotype and site of infection, however, has not been elucidated (Umene et al., 2003) and strain differences alone are unlikely to account for differences in response to infection. The strain of virus together with genetic make up of the host and host immune system determine the response to infection with HSV-1. Thus, the ability of a strain of HSV-1 to cause corneal stromal disease is correlated with its ability to induce vascularisation of the cornea and not with its neurovirulence and nor with the inoculum size (Grau et al., 1989).

3.2. Host factors

A number of physical factors can affect the ability of the virus to establish infection. The tear film contains a number of antiviral agents such as lysozyme, immunoglobulin A, complement and the production of INF-α, β and γ in response to infection. The superficial or outermost layer of corneal epithelial cells, are post-mitotic and incapable of replication. Because HSV-1 replicates best in metabolically active cells (Carr et al., 2001), the intact epithelium thus forms an effective physical barrier to infection. Host genes are involved in innate resistance and acquired immunity to HSV-1. Attempts have been made to map genes affecting innate resistance in animals. For example, in mice the *igh* locus on chromosome 12 confers some resistance to keratitis, the *Hrl* locus on chromosome 6 affects viral replication and reactivation (Brandt, 2005) and loci on chromosomes 10 and 17 are thought to be specific for ocular disease (Norose et al., 2002).

4. Clinical manifestations

Clinical ocular disease develops in less than 1% of the population infected with the virus (Binder, 1977). Whilst lid and conjunctival disease are all manifestations of herpetic ocular surface disease, corneal disease, that is HSK, because of its effect on vision and relation to intraocular disease, takes centre stage.

Corneal scarring occurs in 18–28% with a corresponding reduction in visual acuity to less than 20/100 in 3–12% of cases and less than 6/12 in 10–25% (Liesegang, 1989). In addition up to 42% of patients with bilateral disease have a resultant visual acuity of less than 6/12 (Souza et al., 2003) and 17% of less that 6/60 (Wilhelmus et al., 1981b). Bilateral disease also tends to be associated with a higher incidence of atopy and immune abnormalities (Souza et al., 2003; Chong et al., 2004). Although, somewhat artificial it is helpful to broadly divide herpes keratitis into the corneal layer affected, that is epithelial, stromal and endothelial disease.

4.1. Epithelial keratitis

Early reports showed that the corneal epithelium may be involved in up to two-thirds of cases with ocular involvement (Gunderson, 1936). The earliest lesions are fine or coarse granular spots with epithelial bedewing forming a punctate epithelial keratopathy. Within 12–24 h the cell nuclei become laden with replicating virus and the infected cell swells up prior to releasing the virus into adjacent areas (Edelhauser et al., 1969). This process manifests clinically as a raised dendritiform lesion that displaces fluorescein to produce 'negative staining', but stains with rose bengal (Binder, 1977; Holland and Schwartz, 1999; Liesegang, 1999). This usually progresses leading to destruction of the basement membrane to form a dendritic ulcer, the base of which stains with fluorescein. It has characteristic branching linear shape with large terminal bulbs and swollen epithelial borders. The epithelial borders, usually representing areas of epitheliolysis, stain negative with fluorescein, but can be demarcated with rose bengal or lissamine green (Spencer and Hayes, 1970). Around 22–26% of cases undergo spontaneous cure (Hughes, 1969; Thygeson, 1958). Enlargement of the ulcer may occur, leading to a geographic (amoeboid) ulcer (Fig. 7) in up to 22% of all cases (Wilhelmus et al., 1981a). Ulceration close to the limbus is associated with infiltration of leucocytes from the limbal blood vessels, limbal injection, vascularisation and frequently a more marked anterior stromal infiltrate. In addition, patients with marginal or limbal herpetic ulcers are often more symptomatic and less responsive to treatment (Pavan-Langston, 1975; Wilhelmus et al., 1981a). Although epithelial disease usually resolves, sequaele may ensue such as a persistent

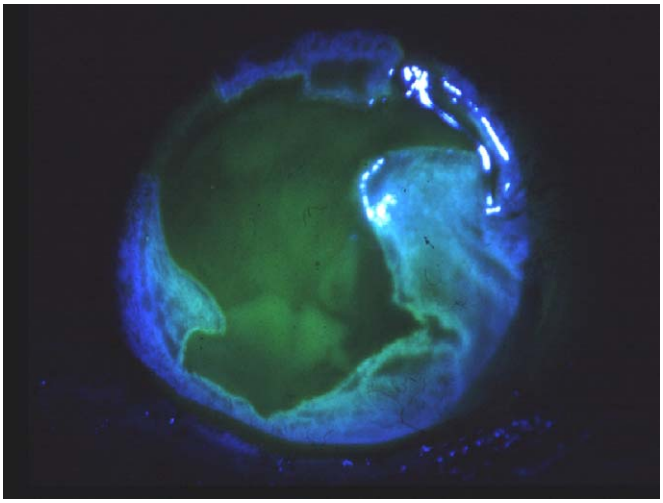


Fig. 7. Geographic ulcer. Note large corneal ulcer stained with fluorescein which is geographic in shape.

punctate epithelial keratopathy, recurrent corneal erosions or epithelial granularity. Some degree of anterior stromal reaction is common underlying the epithelial lesions. A secondary bacterial infection of the ulcer may occur so that many of the features may no longer be recognised. Often overlooked, impairment of corneal sensation may lead to neurotrophic keratopathy, with loss of corneal lustre and an irregularity of the corneal surface. Similarly, punctate epithelial erosions may develop and progress to a persistent epithelial or metaherpetic ulcer with shallow smooth borders of grey, elevated, thickened and rolled epithelium (Binder, 1977; Holland and Schwartz, 1999; Liesegang, 1999).

4.2. Stromal keratitis

Herpetic stromal disease accounts for 2% of initial presentations and 20–48% of recurrent herpetic disease (Darougar et al., 1985; Liesegang, 1989; Norn, 1970). Although, initially thought to represent an immune response to epithelial disease, there is now a body of evidence to suggest that it is due to virus, which has invaded the anterior stroma. Viral invasion of the stroma, either from reactivation of latent virus in the supplying sensory nerves, from direct invasion from the epithelium or from reactivation of latent virus within the stroma together with a marked immune response produces a stromal keratitis.

In a necrotising keratitis, there is necrosis, ulceration, and dense infiltration of the stroma usually with an overlying epithelial defect. HSV-1 antigens and HSV DNA are also present in patients with necrotising stromal keratitis (Holbach et al., 1991). Greyish white homogeneous abscesses with oedema, keratic precipitates, severe iridocyclitis and raised intraocular pressure may develop. Super-additive severe inflammatory response may result in a destructive inflammation leading to thinning and perforation especially where there is a superadded or associated bacterial infection.

A non-necrotising stromal keratitis (previously referred to as an immune keratitis) occurs in 20% patients with chronic or recurrent disease (Liesegang, 1989; Darougar et al., 1985). There may be no history of previous symptomatic epithelial keratitis and the epithelium is usually intact. Stromal inflammation, may be focal, multifocal or diffuse (Wilhelmus, 1987) and there may be an associated anterior uveitis. The inflammation may be chronic, recurrent or recrudescent leading to stromal scarring, thinning, neovascularisation and lipid deposition. Occasionally an immune ring, representing an antigen-antibody complement precipitate, similar to a Wessely ring is seen in the central or paracentral midstroma (Meyers-Elliott et al., 1980). A marginal keratitis or limbitis is usually accompanied by a greater inflammatory or immune response. Stromal neovascularisation may be sectoral or diffuse and frequently occurs in several layers of the cornea (Liesegang, 1999).

4.3. Endotheliitis

HSK may be associated with an endotheliitis. Three phenotypic forms have been identified according to the pattern of endothelial disease: disciform, diffuse and linear. The endothelial function may take many months to recover. It may be very difficult to distinguish between stromal inflammation and stromal oedema secondary to an endotheliitis. A disciform type endotheliitis is the most common form and is seen as a disc-shaped area of stromal oedema in the central or paracentral cornea, usually involving the entire stromal thickness giving a ground glass appearance. Keratic precipitates are present underlying the areas of stromal oedema and a mild-to-moderate iritis is usually present. A diffuse endotheliitis or a linear endotheliitis is much less common. In the former, there is diffuse stromal oedema with underlying keratic precipitates and mild-to-moderate iritis. A dense retrocorneal plaque of inflammatory cells may follow. Linear endotheliitis, appears as a serpiginous line of keratic precipitates, that progresses centrally from the limbus accompanied by peripheral stromal and epithelial oedema (Olsen et al., 1994). Corneal endotheliitis may be associated with raised intraocular pressure, which has been attributed to an associated trabeculitis. This is supported by immunoreactivity for HSV in the trabeculum in these cases (Amano et al., 1999) and by the pattern of stromal oedema, which arises from the peripheral cornea (Ohashi et al., 1991).

4.4. Iridocorneal endothelial syndrome

Iridocorneal endothelial (ICE) syndrome is typically a unilateral condition characterised by corneal endothelial abnormality often associated with corneal oedema, alteration in iris structure (presenting as corectopia) and presumed trabecular endothelial abnormalities leading to secondary glaucoma. Although initially thought to be a developmental disorder, it is now thought to be due to HSV-1. This has been a result of observations that disease onset occurs during the post-natal period and the endotheliopathy resembles that seen in viral disorders with chronic inflammatory cells confined to the endothelial layer (Alvarado et al., 1994). The disease manifestations are thought to be secondary to abnormal endothelial cells (Groh et al., 1999) that can migrate across the trabecular meshwork and iris surface. HSV-1 DNA has been isolated from the aqueous and endothelium of patients with idiopathic corneal endotheliopathy and iridocorneal endothelial syndrome (Alvarado et al., 1994), some of whom have responded to antiviral therapy (Alvarado et al., 1994). In keeping with a herpetic aetiology, the corectopia is predominantly unilateral and tends to be progressive.

4.5. HSK in children

Although, there are relatively few studies, herpetic keratitis in children appears to differ from that of adults.

There is a higher incidence of bilateral disease compared to adults, with reported rates of 10–21% (mean 16%) (Beigi et al., 1994; Beneish et al., 1987; Chong et al., 2004; Colin et al., 1982; Poirier, 1980; Schwartz and Holland, 2000). The inflammatory response in children with stromal keratitis also appears to be greater leading to more common scarring. Chong et al. (2004) found that approximately half of their patients developed a residual corneal opacity. In addition, children are susceptible to recurrences of ocular herpes. Reported studies indicate that approximately 50% (range 33–80%) of children with herpetic keratitis develop a keratitis recurrence within 1–2 years (Beigi et al., 1994; Beneish et al., 1987; Chong et al., 2004; Colin et al., 1982; Poirier, 1980; Schwartz and Holland, 2000).

4.6. Recurrent disease

Recurrent disease has been purported to be associated with several different types of stimuli such as febrile illness, HEDS, psychological stress, menstruation and minor trauma (Binder, 1977; Dawson and Togni, 1976; HEDS, 2000). Earlier reports suggested that the risk of recurrence after a single episode of dendritic keratitis was 18–25%, and 43% in 2 years following two or more episodes (Hughes, 1969; Carroll et al., 1967). In the HEDS study (2001) 35% patients had one or more recurrences over an 18 month period. The risk of developing ocular recurrence without regard to the type of recurrence was strongly correlated with the number of previous episodes. Patients with 4 or more episodes were 2.1 times more likely to have a recurrence in comparison to 1.4 for patients with 2–3 episodes (HEDS, 2001). Previous episodes of epithelial keratitis had no bearing on recurrent epithelial keratitis, but the recurrence of stromal keratitis was strongly related to the number of previous episodes of stromal keratitis (HEDS, 2001). A reasonable estimate of the recurrence rate of HSK after epithelial or stromal disease is approximately 10% per year. The association between age, gender or race and the risk of recurrent HSV keratitis is conflicting with some reporting no relation while others reporting an increased risk with younger populations (Wishart et al., 1987) and male gender (Wilhelmus et al., 1981a).

5. Diagnosis

5.1. Clinically active disease

Although cutaneous lid or corneal lesions are typical, an attempt should always be made to isolate or identify HSV-1. This is important not only because certain other conditions can mimic HSV-1 ocular disease, but also to allow potential strain characterisation and information regarding mutations and superinfection by other strains. Laboratory tests are aimed at—cell cytology, viral antigen detection (immunoassays), viral DNA detection (polymerase chain reaction) and virus isolation (tissue culture).

Cytology is a quick and simple method based on the presence of intranuclear inclusions and multinucleated giant cells. It has, however, a low sensitivity (57%) and specificity and should be used in conjunction with more sensitive tests (Subhan et al., 2004). Enzyme or fluorescence based immunohistochemical (IHC) techniques have good sensitivity but can be difficult to interpret leading so that false positive results become an issue. The polymerase chain reaction (PCR) carries a sensitivity of up to 100% and is very useful for detecting evidence of HSV-1 DNA in the tear film, conjunctival, cornea and aqueous but does not differentiate between latent and infectious virus (hence a low specificity). Competitive PCR is quantitative but at present is time consuming (Farhatullah et al., 2004; Subhan et al., 2004). Real-time PCR, is also a quantitative method, but is quicker and more sensitive. It can provide indirect evidence for virus replication by the number of DNA copies produced and hence can also be used for evaluating the efficacy of antiviral medications (Mengelle et al., 2004). Assessing the yield of the tear film using PCR for different types of HSK, HSV-1 DNA was detected in 100% in epithelial disease and persistent epithelial defects, 57% in disciform keratitis, but was absent from the tear film in silent stromal and endothelial disease (Fukuda et al., 2003). Detection of HSV-1 on corneal samples is addressed below (Section 5.2). Isolation of HSV-1 in culture has a low sensitivity (Subhan et al., 2004), but is still the standard for diagnostic specificity and importantly allows potential strain identification and epidemiological tracing. Cytopathic effects characteristic of virus replication develop with in 24–48 h (Fig. 8). As PCR and IHC detect different components of HSV-1, used in combination an improved diagnostic specificity can be expected.

5.2. Diagnosis of HSV-1 in patients with corneal scars

It is often difficult to determine whether a vascular or avascular corneal scar is herpetic in origin. Sampling of the cornea, either through a corneal biopsy or at the time of

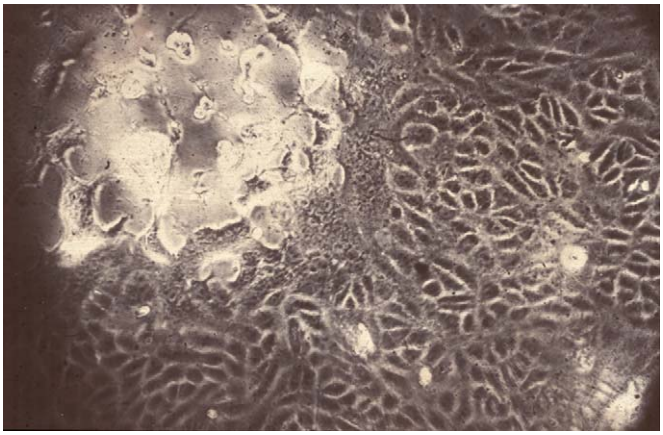


Fig. 8. Isolation of HSV-1 in culture. HSV-1 plaque in monolayer of Vero cells.

corneal transplantation provides tissue for diagnosis. The diagnosis of previous herpetic keratitis is particularly important for patients undergoing penetrating keratoplasty both for prognosis and because of the need for post-surgical antiviral therapy. Although, culture remains the standard for the detection of a productive infection by HSV-1, it is an insensitive technique. This is particularly so for the isolation of HSV-1 from the deeper layers of the cornea particular in the absence of an ulcer. The aggressive use of antivirals may also have an effect on the ability to isolate virus from the cornea. PCR is a sensitive method for detecting HSV-1 DNA in the cornea with a sensitivity of 82% and a specificity of 78%. The longer the period between the last episode of HSK and surgery, the less the amount of virus and the less likely virus will be detected (Kaye et al., 2000). This is in keeping with the results of O'Brien et al., (1998), who found a decrease in the detection of HSV-1 DNA from 100% to 30%, at 4 months post-infection of rabbit corneas. A similar trend has also been noted for the presence of HSV-1 antigen (Fig. 9), which suggests (Section 2.3) that both HSV-1 DNA and antigen have a limited life within the cornea. Although HSV-1 DNA has been found in the corneas of patients with no clinical or diagnosed history of herpetic keratitis, the amount of HSV-1 DNA is much less than in the corneas of patients with a diagnosis of herpetic keratitis. That is, HSV-1 DNA has been detected in approximately a 10–50 times greater amount in HSK than non-HSK corneas. This supports the concept that a critical amount of HSV-1 is required before the infection becomes clinically evident. Similar to PCR, immunohistochemistry (IHC), has a reasonably good sensitivity and specificity for the detection of HSV-1, that is 74% and 85%, respectively (Kaye et al., 2000). The use of PCR followed by IHC if HSV-1 DNA is detected, has a specificity of around 97% for the diagnosis of HSV-1 in corneal samples (Table 1).

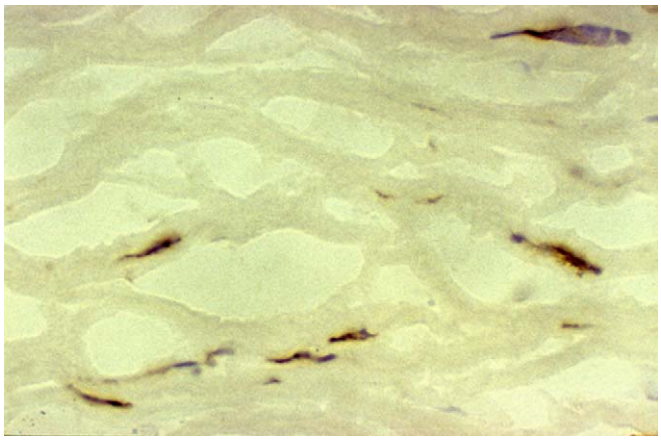


Fig. 9. Immunohistochemistry for HSV-1 antigen in the corneal scars. HSV-1 antibody, DAB and haematoxylin counterstain showing HSV-1 immunoreactivity in the stromal keratocytes.

Table 1
Sensitivity and specificity of PCR, IHC, culture and ISH for the detection of HSV-1

Test	Sensitivity (%)	Specificity (%)
PCR	82	78
IHC	74	85
Both PCR and IHC + ve	68	97
Culture	2	100
In Situ (LAT)	0	—
Strong PCR	28	100
Strong IHC	54	88
Either PCR or IHC + ve	95	63

LAT = Latency-associated transcript

6. Treatment and prevention of HSK

This segment will focus on the use of aciclovir (ACV) and steroids in relation to the developments in the pathogenesis and recurrence of HSK. The bioavailability of ACV as an oral suspension is around 12%. This tends to be less for higher doses due to saturation of absorption, for example, 20% for 200 mg compared to 12% for an 800 mg dose. Intravenous administration provides an 8 times greater bioavailability compared to oral administration. The efficacy of aciclovir depends on the levels over 12 h, so that 250 mg BD provides a similar maintenance level to 1000 mg OD. In terms of dosage, ACV 400 mg five times per day provides therapeutic levels in the aqueous, while 400 mg BD provides prophylactic levels. Fig. 10 shows the pharmacology of aciclovir, valciclovir and famciclovir.

6.1. Herpes simplex epithelial disease

Although most cases of herpetic ulceration will eventually resolve, numerous studies have shown a benefit from using topical antivirals in the treatment of herpetic epithelial or ulcerative keratitis (HEDS, 1996). Improved resolution of bilateral disease has also been found using both topical and systemic antivirals (Margolis and Ostler, 1990). Although, systemic ACV in addition to topical antivirals may have no added clinically evident effect on healing in adults, it has been shown to be of benefit in children where application of topical medication is difficult or compliance an issue (Schwartz and Holland, 2000).

6.2. Herpes simplex stromal disease

It is clear that the use of topical antivirals are beneficial in patients with stromal disease (Bialasiewicz and Jahn, 1984; Sundmacher, 1983; Schwab, 1988). Simon and Pavan-Langston, 1996 reported both a reduction in number (0.14–0.029) and duration (3.08–7.8 days) of recurrences with antivirals. An important issue in the treatment of HSK centres around the use of topical steroids in patients with stromal disease. The Herpetic

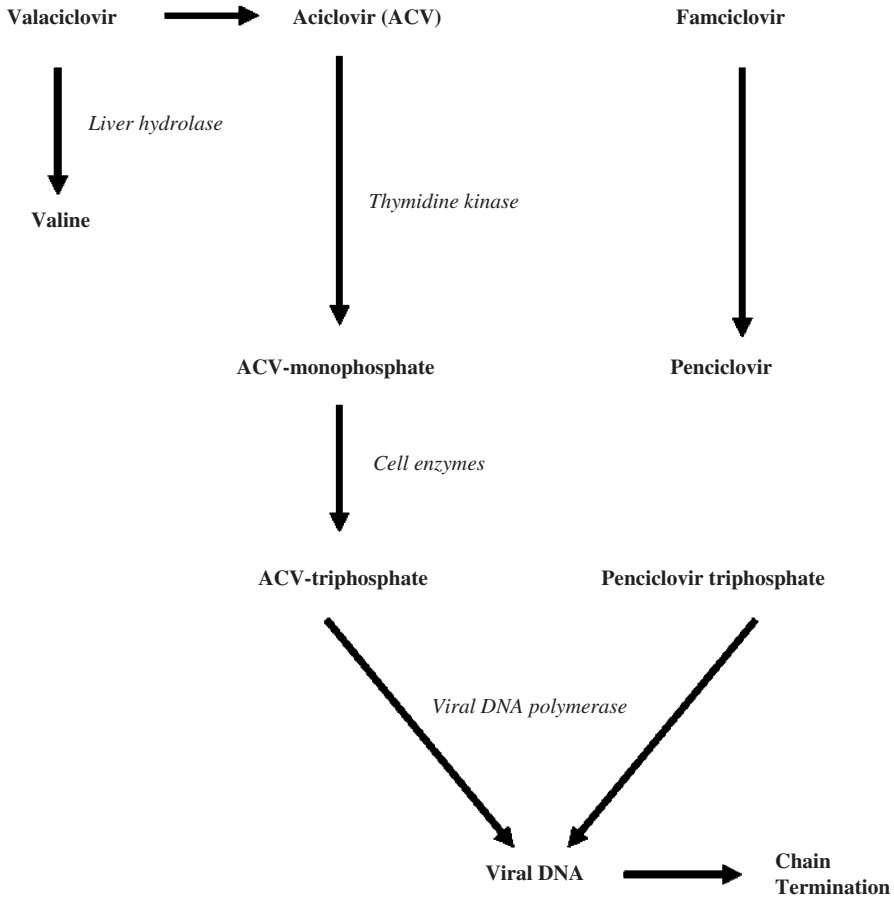


Fig. 10. Pharmacology of Aciclovir, Valaciclovir and Famciclovir.

Eye Disease Study Group (HEDS) showed a benefit in the treatment of herpetic stromal disease using a combination of steroid and antivirals, with a reduced duration of HSK (Barron et al., 1994; Wilhelmus et al., 1994). A faster recovery and an improved outcome have been reported with ACV and dilute steroid than ACV alone (Collum et al., 1983; Oosterhuis et al., 1983). Although, topical steroids and trifluridine shortened the course and prevented progression (Wilhelmus et al., 1994), there was a questionable increase in recurrence in those patients receiving topical steroids. In addition, not all studies have shown a benefit from topical steroids in the treatment of HSK. Protracted tapering when on steroid is a problem with recurrences during the period of tapering (Cunningham, 2000). Steroids may affect or aggravate the severity of the keratitis (Takahashi et al., 1971) and there are reports concerning the development of glaucoma (Souza et al., 2003). Topical ACV without steroids has been shown to suppress stromal inflammation (McGill et al., 1981). What has not been answered, however, is whether initial treatment with antivirals prior to the commencement of topical steroids has an influence or benefit on recurrence. The HEDs study showed no deleterious effect when the introduction of steroids were delayed (Wilhelmus et al., 1994). This is a *very important* issue as the amount of HSV-1 in the cornea may have a bearing on recurrence. As there is good evidence for recurrence of HSK from reactivation of virus within the cornea (Section 2) a reasonable option would be to use topical antivirals alone in at least the first week of treatment followed by the addition of topical steroids in the second or third week.

6.3. Prevention of recurrent HSK

Recurrent HSK is the principal cause of visual loss. Clearly therefore, prevention or reduction in recurrence is likely to have a major impact on visual morbidity. One study (Barney and Foster, 1994) has reported no recurrences of HSK on ACV for 12–15 months compared to 44% recurrence off ACV. In addition, the number and duration of recurrences of epithelial disease decreased with long-term ACV (Simon and Pavan-Langston, 1996). Continued oral ACV reduces recurrence rate of all forms of HSV eye disease. The HEDS (1998) found that oral administration of 400 mg BD over 12 months led to a 45% reduction in recurrence of ocular HSV-1-related disease (32% placebo vs. 19% ACV). In patients with a history of HSK-stromal disease, ACV led to a 42% reduction (28% placebo to 14% in ACV) in recurrent stromal disease (HEDS, 1998). In addition, a benefit of long-term ACV

(mean of 34 months) has also been shown for patients with recurrent epithelial herpes simplex infection (Simon and Pavan-Langston, 1996). Systemic aciclovir in children also appears effective at reducing the incidence of epithelial and stromal keratitis (Schwartz and Holland, 2000) in a manner similar to adults. Recurrences are more common after recent ocular surgery and it would be appropriate to prophylactically treat patients with a history of HSK who have undergone corneal surgery. There is, however, the issue regarding the risk of ACV-resistant mutants following long-term treatment in immunocompromised individuals. The frequency of ACV resistant mutants in the natural virus population has been estimated to be approximately 10^{-4}–10^{-3} (Coen et al., 1982; Honda et al., 2001). In a murine model (Okuda et al., 2004), it was found, however, that ACV sensitive virus tended to replace and suppress the generation of ACV resistant virus during treatment with ACV. This would suggest that conventional ACV therapy in immunocompromised hosts might not induce ACV-resistant HSV.

6.4. Recurrence after keratoplasty

Once a visually disabling corneal scar occurs options for improving vision are limited to surgery either in the form of penetrating or lamellar keratoplasty or rarely photokeratectomy. One of the major problems following penetrating keratoplasty for HSK is the development of recurrent disease in the new cornea (Fig. 11) and secondly corneal transplant rejection following or concurrent with recurrent herpetic disease. Recurrence of 27% in first year was reported (van et al., 1995, 2003), 18% by 2 years (Cobo et al., 1980), 12% after 3 years (Fine and Cignetti, 1977), 15% after 2 years (Langston and Pavan-Langston, 1975) and 24% within 1 year (Moyes et al., 1994). The recurrence of HSK after in penetrating keratoplasty therefore, varies from 12% to 27% compared to 10% per year following HSK. Conversely, the incidence of newly acquired HSV keratitis after penetrating keratoplasty is 14 fold higher than in the population (Remeijer et al., 1997). This increase may be due to reactivation of latent virus, transmission via the tear film, or transmission from virus either latent in the donor cornea or carried via the donor cornea following reactivation in the trigeminal ganglion (Remeijer et al., 1997, 2001, 2002). HSV-1 DNA has been detected more frequently in the corneas of patients undergoing repeat corneal transplantation, and has been suggested as a possible role for HSV-1 in graft failure (Kaye et al., 2000). Nicholls et al. (1996) and Openshaw et al. (1995) were unable to elicit disease in uninfected animals by transplanting corneas containing HSV-1. It is likely therefore, that HSK post-transplantation arises from host HSV-1. Antiviral prophylaxis translates to treatment post-operatively. The use of both topical and systemic antivirals has led to a significant reduction in post-transplant recurrence of HSK. Several studies have supported the use of antivirals in the post-operative period and during episodes of transplant rejection. Most episodes of HSK will occur within the first year following penetrating keratoplasty and so it would appear reasonable to treat prophylactically for at least 1 year post-operatively. Van et al. (2003) found that recurrent disease while on 1 year of aciclovir was 9% compared to 27% in the placebo group. Similarly, Foster and Duncan (1981) noted 6% recurrence using topical anti-virals after 2 years and Moyes et al. (1994) reported a 24% recurrence without versus 11% on topical antivirals. This antiviral effect has been documented in animal studies as well. For example, Beyer et al. (1989) reported a reduction in the shedding of HSV-1 in the tear film from 82% to 0%, ulcerations from 82% to 10% and stromal keratitis from 56% to 12% in rabbits after PK.

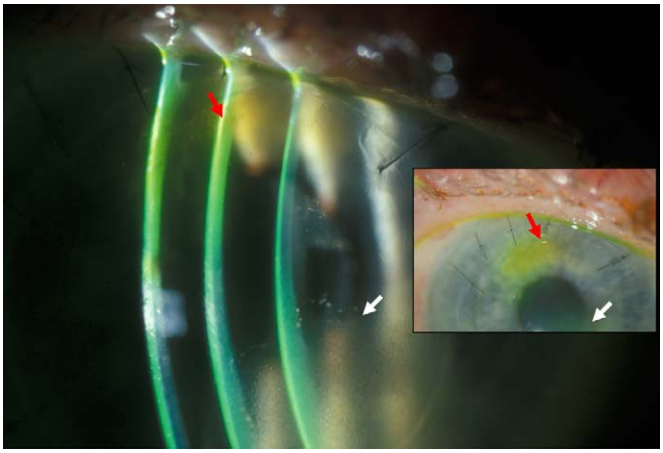


Fig. 11. Recurrence of HSK in a corneal transplant. Note the recurrent corneal ulcer at the interface (red arrowheads) and line of keratic precipitates with inferior corneal oedema (white arrowheads) in a patient who had a corneal transplant for HSK.

7. Summary and future directions

HSV-1 may enter the ocular surface either directly through droplet spread, the 'front door' route (Kaye et al., 1992; Kaye and Baker, 1996) or indirectly via neuronal spread from a non-ocular site, the 'back door' route (Tullo et al., 1982). HSV-1 may persist in the cornea but this appears to be time dependent (Kaye et al., 1992, 2000). Superinfection with a different HSV-1 strain may also occur (Remeijer et al., 2001, 2002). During latency the virus appears as a circular episome associated with histones with active transcription only from the region encoding LAT (Wang et al., 2005) (Kubat et al., 2004). The latter is implicated in neuronal survival, anti-apoptosis, virulence, suppression of transcription, establishment of and reactivation from latency. Visual morbidity usually results from recurrent disease through corneal scarring, thinning and neovascularisation. Recurrent HSK may arise from reactivation of virus either within the cornea (Polcicova et al., 2005) or following anterograde spread from trigeminal ganglion (Shimeld et al., 1989). The latter is not, however,

necessary for the development of recurrent HSK (Polcicova et al., 2005). Corneal neovascularisation is an early and important event in the development of stromal disease, which is due to an immune response to virus within the cornea. The ability of the strain of HSV-1 to cause corneal stromal disease correlates with its ability to induce corneal vascularisation. Evidence now suggests that HSV-1 infection disrupts the normal equilibrium between angiogenic and anti-angiogenic stimuli leading to an angiogenic switch (Sottile, 2004). TSP 1 and 2, matricellular proteins involved in wound healing, are potent anti-angiogenic factors and appear to be one of the key players. The effect of HSV-1 on TSP secretion is inversely correlated to the production of the immediate early ICPs (Hiscott et al., 1999; Choudhary et al., 2005). This appears to be a specific effect on TSP 1 and 2. The VHS and ICP0 genes would be reasonable targets to elucidate the molecular events leading to a suppression of TSP 1 and 2 following HSV-1 infection. Treatment with angiogenesis inhibitors from the outset of infection in animal studies, significantly reduces the severity of HSV stromal keratitis and vascularisation (Zheng et al., 2001b). The addition therefore, of TSP1 and TSP2, or their angio-active elements (Tolsma et al., 1993; Vailhe and Feige, 2003), in the early stage of the disease may enhance existing or provide alternative anti-angiogenic therapy in HSK where stromal vascularisation is related to disease severity.

Acknowledgements

Part of our research was funded by the Royal College of Surgeons of Edinburgh.

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