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The ultimate terminus of evolutional symbiosis leaves a flaw: Blinding herpetic stromal keratitis

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Abstract

Herpes simplex virus (HSV) infects epithelial cells and establishes lifelong latency in neuronal nuclei of the regional ganglion. Although active replication of HSV can be treated effectively with anti-HSV drugs, control of the reactivated HSV in the regional ganglion is a difficult task: Reactivated HSV in the trigeminal ganglion (TG) comes down via axon and infect the cornea Some patients eventually develop vision threatening herpetic stromal keratitis (HSK) that is the second leading cause of corneal blindness after trauma. Corneal herpes continues to cause blindness in developed countries. In this review, we describe initial attachment of HSV, entry and infection at peripheral epithelial cells, establishment of latency in neuron, reactivation and egress, corneal infection and immunopathology of HSK. We also discuss prevention and treatment of the disease.

Herpes simplex virus (HSV) is a neurotropic virus

Worldwide, more than 1 billion individuals carry a latent HSV-1 in sensory neurons, predominantly in the trigeminal ganglion (TG) [1,2]. Primary infection of HSV-1 occurs in the orolabial mucosa without any symptom [1-3]. When overt disease is elicited, infected epithelial cells are eliminated rapidly by the host immunity (4). When the infected area is innervated with the trigeminal nerve, HSV becomes latent in the TG [5,6]. Axons consist of microtubules oriented toward the plus-end terminus. Entering viruses are transported retrograde via microtubules by dynein while kinesin conveys cargos anterogradely [7-9]. This initial movement develops lifelong HSV-1 latency in nerve growth factor (NGF) reactive A5+ neurons while HSV-2 in contrast is latent in KH10+ neurons [10,11]. The majority of infected neurons survive without apoptosis or necrosis so that sensitivity of the corneal surface remains unchanged during HSV latency in TG [12]. Reactivated HSVs are frequently detectable in tears, saliva or in secretions of the genital tract. These are major routes of virus spread and the source of primary infection of HSV [13]. A small population of people have developed recurrent diseases such as ulcers in the oral mucosa or lips, dendritic keratitis and genital herpes even if they were immunologically educated [14]. After repeated episodes, corneal herpes eventually develops to an immune mediated herpetic stromal keratitis (HSK). HSK is still an important cause of blindness in developed countries [15].

Clinical ocular HSV infection

The eye is an immunologically privileged site [16]. It strives to maintain transparency of the cornea to preserve the pathway of light to the retina. Microbial invasion on the corneal surface is prevented by washing out infectious agents with tears containing inhibitors such as lactoferrin, lysozyme, secretory IgA plus complement, type 1 interferon and suppressing factors on the growth of new blood vessels. These sustain inflammatory cell infiltrations [17-20].

However, primary ocular HSV-1 infection does occur. It causes acute kerato-conjunctivitis with hyperemia, edema and swollen lymphoid follicles in the conjunctiva which is often indistinguishable from acute adenovirus infection [21]. Corneal ulcers extend to dendritic figures. It may spread to cause a geographic ulceration [21, 22] (Figures 1 and 2).

Reactivated virus causes unilateral corneal herpes, although rarely it can occur bilaterally [22-25]. The cornea tries to maintain status quo, thus a few patients develop stromal haze namely herpetic stromal keratitis (HSK) after repeated episodes of recurrences [26]. HSK has been studied using the patient's excised corneas obtained at corneal transplantations [26] and/or in experimental animal models [28-34].

Experimental HSV infection in vivo

To infect the mouse or the rabbit cornea, a virulent RE or Mckrae strain of HSV [34,35] is instilled onto the scratched cornea. In mice, HSV replicate for 4-6 days in the cornea, then the virus disappears. Following a short quiet period [47], inflammatory cells infiltrate the corneal stroma at 8 to 10 days post infection (PI) with rapid neovascularization of the entire cornea proceeding to the complete loss of clarity of the visual axis. This immune-mediated pathogenesis has been extensively studied as a model of HSK [47, 48]. However, no experimental animal model of HSK has been developed as an outcome of repeated recurrences of reactivated HSV [32-34]. Two to three days PI of HSV on the mouse cornea, progeny viruses are transported to the TG via retrograde axonal flow [5,7,8]. After a short period of active growth in the TG, HSV establishes lifelong latency in the neurons with occasional episodes of reactivation [36]. In mice, spontaneous reactivation is rare [37-39]. Stimuli such as UV irradiation [40], heat [41], tattoo [42], chemotherapy and steroid [43], epinephrine iontophoresis [44], T cell and γIFN removal [45], psychological stress [46] etc. have been used to reactivate HSV, however, results are inconsistent in mice.

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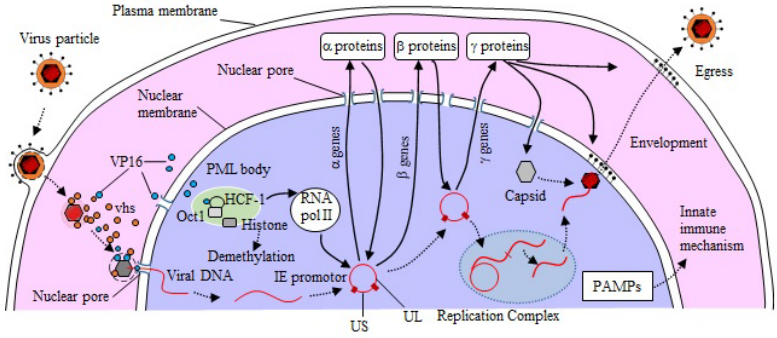

Figure 1. Entrance and proliferation of HSV

HSVs enter epithelial cells following the regulated processes: attachment, binding fusion, and penetration into cytoplasm. Entered capsid and tegument proteins like VP16 trafficked toward the nucleus. Through the nuclear pore, viral genomes are injected into the host cell nucleus. Immediately, viral DNAs associate with host heterochromatin in the host cell nuclei. After de-repressing process, HCF-1, Oct-1 and VP-16 start replication processes of HSV, namely immediate early α, early β and late γ genes transcriptions. Newly synthesized genome forms a rolling circle and cleaved into equimolar unit. It is packaged into a newly made capsid. Mature enveloped viral particles are bud from the cellular membrane.

VP-16: virus protein 16, HCF-1: host cell factor 1, vhs protein: virion host shut off protein, PML body: promyelocytic leukemia body UL and US: unique long and short region, PAMPS: pathogen associated molecular patterns

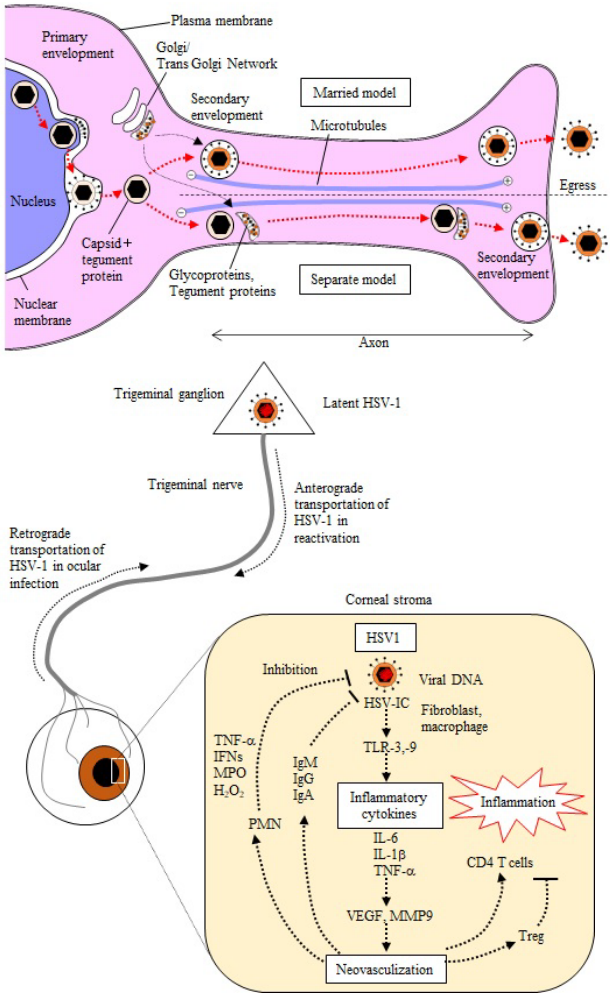

Figure 2. Egress of HSVs via the anterograde transport on axonal flow

Two egress processes have been observed. HSV capsids enveloped at inner nuclear membrane where HSV capsids obtain tegument proteins VP 16, UL11 and glycoproteins and released into the cytoplasm. They de-envelope at the outer nuclear membrane and the naked capsids released in the cytoplasm. They entered cytoplasmic membrane and mature with a secondary envelope. This mode of maturation and egress is called a married model. Another mod of egress is a separate model. Naked capsids with tegument protein are transported through the axon. Envelope with viral glycoproteins is transported separately and these components are assembled at the sites of egress where epithelial or fibroblastic cells are adjacent of nerve endings. Both models are observed, and the married model may be the dominant one. Long distance transport is depending on the kinesin motors. Microtubules composed of head-to-tail association of α and β tubulin transport virus to the cellular periphery. Reactivated virus transported to the peripheral tissues. In the cornea HSV DNA or viral proteins are detected but they are reactivated virus in trigeminal ganglion transported via axonal F flow.

The rabbit establishes HSV latency in the TG after acute corneal infection. Reactivation is easier than the mouse; however, immunological analyses of the results in the rabbit are difficult [48]. Guinea pig is a model of genital HSV-2 infection with spontaneous reactivations [49]. When attenuated HSV-2 is inoculated intra-nasally, educated CD4+T cells migrate to the vaginal mucosa and secretory anti-herpes IgA is produced locally by activated B cells [49,50]. Preventive effects are being investigated in several laboratories [51]. The effects of this immunization for corneal herpes has yet to be studied.

Entry of HSV into Cells

Viral envelope glycoproteins gB, gC, gD, gH and gI attach onto the plasma membrane [52-54]. These glycoproteins fuse at the lipid rafts and dynamin on host cell membrane [56]. HSV-1 gB and gC (HSV-2 ligands are gB and gG) adhere to the cell surface glycosoaminoglycans [52-55]. Following entry steps, three gD receptors for HSV-1 [56]: herpes virus entry mediator (HVEM) [58,59]; nectin-1 and nectin-2 [60,61]; and 3-0-sulfated heparan sulfate [62]; are required [56,57]. Nectin-1 is expressed on the corneal epithelium, conjunctiva, lens epithelium, ciliary body, iris, choroid and retina. PTLR α is an inhibitory receptor paired with an activating PTLR β receptor. The cellular ligand for both PTLRs is D99 expressed on activated T cells [63-65]. It deactivates NK cells and prevents HSV infected cell lysis. Glycoproteins gD and gB bind to cell surface receptors: non-muscle myosin IIA (NM-IIA) [63]; paired immunoglobulin-like type 2 receptor α (PILR α) [64, 65]; myelin associated glycoprotein (MAG) [66]; with membrane fusion of gH [57]. When NMHC-IIA, a subunit of NM-IIA [67], is expressed on the cell surface, gB is attached and fused with the cell membrane. NMHC-IIA expression on the corneal and conjunctival cell and NMHC-IIB on neuronal cell are scarce [67,68], however upon HSV entry, they are protruded into microvilli, filopodia [69] etc. by muscle myosin movement [68]. The corneal surface is washed with microbial inhibitors in tears [17-20] by constant blinking. In this environment, NM-IIA offers a way for the HSV infection to the cornea [67]. HSV also enters cells by endocytosis [70]. HSV-1 gH/gL binds to $\alpha_v\beta_3$-integrin [56,71] and enters HeLa cells and CHO cells by endocytosis on lipid rafts [56]. HSVs are degraded by lysosomal enzymes released in endosome. Macrophages phagocytize HSV when pseudopods are extended by activated TLR2. HSV DNA in the phagosome are degraded by lysosomal enzymes and recognized by TLR 3 and 9 [28,72-75]. Signals of activated TLR3 and 9 are transduced to the NF-κB [76]. When HSV passes through the cytoplasmic membrane, activated Ca++ ion [77], necessary for the transport of viral capsids to the nuclear pore, flows into the cytoplasm from the endoplasm [78]. Nearly 20 tegument proteins are dispersed into the cytoplasm. Inner tegument proteins complexed with capsid are transported to the nuclear pore by the motor dynein in microtubule [78]. Polyamines in the virion neutralize the negative charges of the viral DNA [79]. It is packed tightly in the capsid where the inner pressure reaches about 20 atmospheres [80]. Host nuclear factor importin β, nuclear pore complex proteins and UL36 serve to dock HSV capsids to the nuclear pore [81]. Viral genome is ejected into the nucleoplasm with the high inner pressure [80].

HSV infection and proliferation

In the nucleus, HSV DNA is linearized and circularized [82] before the viral protein production (Figure 1) [83,84]. When replicating, it forms a rolling circle and extended genomes are packaged into a newly made capsid after cleavage into equimolar monomeric units [84]. In the epithelial cell, 103-104 virus particles are produced in 8-10 hours [85]. Immediately after the entrance into the nucleus, promoter regions of HSV genome; α (immediate early), β (delayed early) and γ (late) gene associate epigenetically on the methylated histone H3-Lysine 9 and 27 host heterochromatin [86,87] .

Sixty eight percent of the HSV-1 genome consists of (G+C) [88] Speckled protein of 100KDa (Sp100) binds hypo-unmethylated CpG islands [88-94]. In addition, the HSV genome triggers innate anti-DNA responses with interferon α or γ by promyelocytic leukemia (PML) protein bodies in nuclear lamina or nuclear domain (ND10) [95,96] locating in the inter-chromosomal spaces or near the nuclear membrane. Upon HSV infection, ND 10 constituents are degraded, dispersed [97-101] and provided places for the latency or replication of HSV genome [102,103,104]. RING finger domain of ICP 0 [99] confers E3 ubiquitin ligase activity in its N-terminal region [100] and triggers the proteasome dependent degradation of PML and Sp100 [101]. PML proteins contain repressive cofactors Sp100, Daxx and ATRX [99]. Upon sensing foreign DNA, nuclear interferon inducible protein 16 (IFI 16) binds to the transcriptional activators of viral genome and links to the heterochromatic suppression of HSV genome [104-106]. Depletion of IFI16 in epithelial cells or fibroblasts results in histone modifications of decreased H3K9me3, increased H3K4me3 of ICP4 promotor and increased expression of IE genes [105,107]. They also increased RNA polymerase II loading on IE genes in HSV infected cells [108]. Corepressor element-1 silencing transcription factor (CoRest)/ REI-silencing transcription factor (REST) repressor complex consists of histone deacetylase (HDAC) and CoRest binds HDACs and Rest [109]. This complex functions to repress neuronal genes in non-neuronal cells [110]. CoRest interacts ICP0 which colonizes with ND10 in the nucleus and blocks the repressive action of HCLR [109,111]. In epithelial cells, ND10 disassembles in a few hours and dispersed in the nuclear microenvironment [112]. These heterochromatin repressions are counteracted by viral (VP16 and ICP0) and host cell factor 1 (HCF1), lysine specific demethylase1 (LSD1), octamer protein-1(Oct-1), specificity protein 1 (Sp1) and GA binding protein (GABP) transcription factors. LSD1 plays a key role in the de-repression of α genes [113]. Alpha HSV-1 gene promotors contain binding sites for Oct-1, VP16 and a cellular protein designated HCF1 [114-117]. Oct-1 and VP-16 bind to the promotor domains. VP16 assembles HCF1, Oct-1 and lysin specific demethylase 1 (LSD1) changes heterochromatin to euchromatin. LSD1 is recruited by VP16 from cytoplasm to nucleus. ICP0 is dispersed in the cytoplasm. On productive infections of HSV-1, after entering viral DNA into nucleus, HCF1, Oct-1 and other transcription factors reduce heterochromatin in an hour and activate transcription of α-genes [118]. With these de-repressing processes, viral immediate early genes (α genes: ICP-0, -4, -22, -27, -47, Us1.5) start to be transcribed. After α gene expression, β1→ β2 genes and γ1→γ2 genes are expressed in cascade fashion [1,119]. β genes produce enzymes for the DNA replication and γ genes make structural proteins and assembly of infectious viruses [1]. Immediate early protein ICP-0 and ICP-4 activate β and γ gene expression [1]. ICP-0 is related to the transcription, interferon response, cell cycle and degradation of ND10 [120]. It also plays a key role of reactivation from latency [120]. ICP-27 inhibits mRNA translation by disrupting a splicing of RNA and helps transport viral mRNAs to the nucleus [121]. ICP-47 interrupts TAP conjugation of viral antigens with MHC at the endoplasmic reticulum and hinders transportation to the cell surface. Hence CD8+T cells can not recognize viral antigen and do not activate [122].

Establishment and maintenance of latency in neurons

When HSV enters the ganglionic neurons, viral lytic genes are repressed associating with the host cell heterochromatin and latency

associated transcripts (LATs) promotor and enhancer bind to the euchromatin [92,123]. To be functional in neurons, HSV DNA requires several days more than in epithelial cells for several reasons. First, the pool of histones is much less in the nucleus of sensory ganglionic neurons. Second, most of the HCF-1 is retained in the cytoplasm and is scarce in neuronal nuclei. Third, after the virus passes through the cytoplasmic membrane, envelope protein VP16 remains in the cytoplasm [114,124-126]. LATs expression in neurons reduce lytic gene expression during the acute phase of infection [127,128]. These characteristics of neurons contribute to HSV latency in neuronal cells. Some infected TG neurons produce progeny HSVs but do not cause apoptosis or necrosis [129,130]. The majority of infected ganglionic neurons survive harboring the circular HSV genome as episomes [69,131]. All these outcomes are supported by epigenetic regulations and innate or acquired immune responses [132-135]. Functioning neurons in TG are detectable by sensing stimuli on the corneal surface [12]. In mice, HSV DNAs in the TG are detected by in situ hybridization. They are distributed to neighboring cells via dendrites in all three branches of TG several days after corneal infection [136]. Any ganglionic neurons may harbor up to a thousand copies of HSV DNA (137). These results indicate that infiltrating CD8 T cells don't induce apoptosis in TG [133-135, 137,138]. Release of exosomes from neurons containing mRNA, microRNA (miRNA), viral proteins, HSV DNAs and/or infectious viruses might contribute wide spread of HSV [139-140]. Neutrophils break up these multiple apoptotic bodies containing live viruses. They are phagocytized by macrophages and spread virus to neighboring ganglionic neuronal cells [141,142].

During latency, HSVs transcribe stable two major antisense RNAs, latency associated transcript (LATs), from the unique region of the viral genome. HSVs also transcribe minor LAT only detectable with sensitive methods [138-139]. LAT is an 8.3kb primary transcript, which is spliced into stable 1.5 and 2.0 kb major LAT introns, as well as a 6.3 kb minor LAT exon [143,144]. LATs stabilize latent HSVs [145,146] and downregulate genes required for a lytic infection. Discovery of the gene regulation by small RNAs (sRNAs) was swiftly extended to the study of herpesviruses. HSVs encode many miRNAs within or adjacent to the LAT locus. Two sRNA1 and 2 are encoded in the first 1.5kb LAT. They inhibit productive infection in tissue culture [147,148]. sRNA2 suppresses ICP4 and increase HVEM expression on neurons [149] without affecting cognate mRNA levels [148]. In addition, LATs transcribed twenty-seven miRNAs [1,149-152]. Eleven miRNAs are expressed in the related region of LATs and their functions have been studied in neurons and epithelial cells [149,150]. Primary LAT gene encodes seven miRNAs, while the eighth is derived from a transcript upstream of the LAT promotor [153]. Less abundant potential miRNAs originating from LAT region are identified by deep sequencing. Most of these miRNAs suppress immediate early gene expression by their antisense sequences [152]. Six miRNAs: miR-H2-3P, H3-3P, H4-3P, H5-3P, H6-3P and H7-5P accumulate in latently infected neurons [149,150]. miR-H2 is complementary to ICP0 mRNA and regulates ICP0 expression [149,153]. It promotes latency by globally repressing lytic gene expression [154]. However recent results suggest that H2 is less effective in inhibiting expression of ICP0 than neuronal host miR-138 which inhibits lytic gene expression in ganglia by targeting ICP0 mRNA [155,156]. Mutated miR-138 target sites increased α-gene expression in the infected eye and TG [155, 156]. H-4 downregulates neurovirulent ICP34.5 expression [157]. Except H4-3P, all miRNAs are expressed in infected cells in which progeny viruses are produced. miR-H8-5P, H15, H17, H18, H26 and H27 accumulate during reactivation (158). miR-H3-3P, H13 and H27 enhance growth of HSV [158]. H5-3P and H27 are produced without viral protein synthesis similar to the viral α-gene products [158]. The synthesis of H1-5P, H3-5P, H6-3P, H7-5P, H16 and H26 require prior protein synthesis like those of viral β and γ-mRNAs [158]. In mouse studies, miR-155, a host's miRNA, controls HSV latency and prevents spread of HSV from TG to the brain. It also suppresses zosteriform spread of skin lesion by intradermal HSV infection [159] LATs have been strongly implicated in the global control of HSV gene expression during latency through the post-translational modification (PTM) by methylation on histone H3 associated with viral promoters [160,161]. As described in the previous section, IFI16 is an innate sensor of the foreign DNA in the nucleus. In the cytoplasm, cGAS enzyme binds STING (Stimulator of Interferon Genes) and activates the IRF-3 signaling pathway [162-164]. STING and cGAS sense HSV DNA as foreign and synthesizes cyclic guanosine monophosphate-adenosine monophosphate (cGAMP). Together with IRF3 and IFI16, cGAMP induces IFN-β in HSV infected cells [162-164].

When HSV infected, elicited stress leaks mitochondrial DNA (mtDNA) into the cytosol [165]. cGAS and STING sense the situation and elevate a type 1 interferon response [164]. Foreign DNA and viral proteins induce expression of IFNs, inflammatory cytokines and chemokines that recruit inflammatory immune cells to TG [132, 166]. Neutrophils are scarce in TG but monocyte, mast cells, macrophages, γδT cells are infiltrated and surround infected neurons [167,168]. γδT cells induce IFN-γ and activated macrophages secret NO and TNF-α [169, 170]. These inhibit viral growth. Eventually, circulating CD8+T cells and CD4+T cells, but not tissue resident T cells, accumulate in inflammatory sites in TG [133]. Neurons usually do not express MHC-I; however, in TG, neurons contain latent HSV [171]. These neurons express small amount of viral proteins such as gC, $gB_{498\text{-}505}$ with MHC-I [133,135]. $CD8^+$T cells recognize them with direct contact. In TG, LAT positive neurons are surrounded by primed $CD8^+$, T cells at the periphery [170-172]. LAT positive neurons are protected from apoptosis by granzyme B released from $CD8^+$T cells [135,173]. However, recent evidence suggests that $CD11c^+$, $CD8\alpha^+$ monocytes play the major role to establish HSV latency and reactivation in neurons [174]. $CD8^+$ T cells are eventually exhausted with programmed death-1 (PD-1) expression with increased latency [175]. In neurons, autophagy is another important defense mechanism [176].

Reactivation and egress of the reactivated virus

Various stimuli in TG shift a micro-environmental balance to a favorable one for viral α gene expression rather than for cellular repression [160,177]. An in vitro study of HSV reactivation indicates that the initial step is induced following a removal of nerve growth factor (NGF) in culture medium [178-180]. *In vivo*, tyrosine kinase A (TrkA) receptor activates PI3-K (phosphatidylinoisitol 3-kinase) p110 alpha catalytic subunit with NGF [181-183]. When NGF is withdrawn in culture medium, 3-phosphoinositide-dependent protein kinase-1 (PDK1) is recruited to the plasma membrane and phosphorylates serine/threonine kinase Akt [179,180,183]. The NGF removal inactivates mTORC-1, blocks 4E-BP1 phosphorylation, suppresses cap-dependent mRNA translation and induces HSV-1 reactivation [181]. External stimulations sensed by trigeminal nerve endings distributed in orolabial epithelia or ocular surface are immediately delivered to the TG neurons. When latent HSV DNAs in neurons sensed the reduced level of NGF, initial steps taken by silenced HSV DNA are different from a well-ordered cascade fashion replication [184,185]. They are controlled by infiltrated $CD8^+$ T cells, NK cells and γδT cells [135,186,187]. IFN-γ is produced by them, then HSV replication is halted and there is a return to the latency (187). For the initial 24 hours, VP16/UL48

translocates from the cytoplasm to the nucleus [124,188] and guides Oct-1 and HCF-1 [182,189]. Only in a small number of neurons, ICP0 and ICP4 are expressed and degrade ND10 [190]. Progeny virions are made in cascade fashion and they are transported to the axonal termini [182]. Virus gene transcription does not follow with any specific kinetic schedules seen in the epithelial cells, but reactivated virus released to the peripheral tissues at 14 to 48 hours after stimulation signals received [182]. Most of the released viruses don't produce overt disease. They are shed silently in the tears, nasal or genital secretions and/or saliva, but they may be a source of virus spread [191].

Following reactivation, newly synthesized concatemeric virus genome is processed as a rolling circle mode described in the previous section [1,192]. Reactivated virus spreads from TG to peripheral tissues via axons. As ganglionic neurons are bipolar cells, HSV can spread in both directions [136]. It can cause encephalitis in rare instances [8]. Initial innate immunity and subsequent specific immune responses prevents viral invasion in the brain but latent HSV can be detected in the neurons in the brain [193].

Packaged viral nucleocapsids in the nucleus bud through inner nuclear membrane where they obtain a primary envelop (Figure 2) [194]. During this primary envelopment, they have obtained tegument protein VP16, UL11 and membrane glycoproteins [195,196]. Egress of viral capsid through the nuclear pore by fusion of gB with outer nuclear membrane is dependent upon the viral proteins UL31, UL34 and Us3 kinase [197]. At the outer nuclear membrane, capsids de-envelope and naked capsids are released into the cytoplasm [197]. The virions are processed for assembly and maturation in the cytoplasmic transport vesicles where capsids coated with tegument, then obtain a secondary envelope containing markers of trans-Golgi network (TGN) and viral glycoproteins [198]. Capsids move toward transport vesicles. UL36, UL37 and Us11 in the tegument interact with kinesin motors [199,200]. Hollow 25nm diameter microtubules composed of 13 protofilaments are tied in a head-to-tail association of α-tubulin and β-tubulin in the axon and used for a long distance transport of virus to the periphery [201]. They have a 'plus' end oriented towards the cellular periphery [202,203]. The cargo of virus particles egresses from axons with three viral envelope proteins gI, gE and Us9 [204]. Currently there are two models of transportation (Figure 2) [205]: "The married model" tells us that virus particles are assembled and enveloped in the neuronal cell bodies before entering axons and transported to egress at the peripheral distal ending [5]. "The separate model" indicates that naked capsids with tegument proteins but without envelop are transported through the axon. Envelope with viral glycoproteins are transported separately and these components are assembled at near the sites of egress [206]. Both models are observed, and the married model may be the predominant one. Virions are released into an extra-neuronal environment by fusion of transport vesicles with an epithelial cell or fibroblast plasma membrane. As described in the first section of this review, most of them cause asymptomatic shedding.

Immuno-pathogenesis of corneal herpes

In HSK, infiltrated cells release angiogenic factors, EGF via IL-6, Robo 4, MMP-2 and MMP-9. Leaky new vessels grow from the limbus to the cornea l center. Inflammatory cells infiltrate via the new vessels. Visual acuity is severely hampered by the cloudiness of the corneal stroma. This is called HSK [24-28] (Figure 3).

The immunopathological nature of HSK is recognized by a ring-shaped haze where IgM, IgG and IgA are deposited together with virus particles [207]. Viral antigens deposited in keratocytes induced anti-HSV antibodies plus complement deposition or antibody-dependent cell mediated cytotoxicity (ADCC) with inflammatory cellular damage [17,207-209]. The antivirals combined with steroid keeps the affected cornea clear. This supports the immunological nature of the disease [210]. Rabbits showed immune complexes play a role in the pathogenesis of HSK [207]. In HSK, aberrant Th1 type responses mediate chronic immuno-inflammation [25,31,211]. When the mouse corneal epithelium is inoculated with HSV-1, it occasionally invades the upper corneal stroma until 4–5 days post infection (PI) [35]. During this initial phase of infection, cytokines (IL-1α, IL-1β, TNF-α, IL-6, IL-12, IFN-γ), chemokines (IL-8, MIP-1α, MIP-1β, MCP-1, MCP-2, RANTES.), and other mediators such as nitric oxide, COX 2, prostaglandin E2 are produced either by resident corneal cells or infiltrating cells [31,47,76,212-215]. Neutrophils and macrophages are detected in the active lesion. After a 3-4 days interval, robust immuno-inflammatory cells infiltrate in the stroma [31,35]. This infiltration consists of mainly CD4 T cells [47,216], which recognize HSV tegument proteins UL21 and UL49 [217,218]. CD4 T cells, NK cells and Langerhans cells infiltrate via new vessels [31,219-222]. Nude or SCID mice [223,224] do not develop typical HSK. Regulatory T cells (Treg) were found to improve clinical signs in murine chronic HSK [225]. When regulatory CD4 T Foxp3+ cells are dominant over the CD4 Th1 cells, HSK lesion remains mild [226,227]. In current models, herpetic keratitis has been induced in the naïve mouse by the inoculation of a large amount of virus in the scarified cornea. However, the frequent virus reactivation does not occur in animal model of HSK [32,34,35,228]. Instead, in humans, repeated or continual recurrent episodes of virus reactivation from the ganglion are observed in a person who has sufficient innate and adaptive immunity. Further investigation requires to answer what viral or immune molecules drive stromal keratitis in the chronic phase without active viral replications in the current mouse model.

Human corneal buttons harbor HSV-1 DNA

Large amounts of bioactive CpG motifs in HSV DNA can induce NO and Th1 type cytokines like IL-6, TNF, and IFN- in vivo and in vitro [81,205,224]. Macrophages and dendritic cells recognize them by TLR-9 expressed on the endosome [213,229]. In the human corneal button or experimental mouse cornea, HSVDNA has been detected by in situ hybridization or conventional PCR [26,224]. Real time PCR demonstrated HSVDNA persistence in the corneal buttons obtained from HSK patients [26]. Viral antigen and antibody complexes are deposited in human HSK [207]. Toll-like receptors (TLRs) recognize pathogen associated molecular pattern (PAMPs) of HSV and trigger initial host responses [224,230-232]. TLR-3 is expressed in the endosome and the surface of corneal epithelial cells and fibroblasts and recognizes double stranded RNA produced during the process of HSV replication [233,234]. TLR-9 expressed in the endosome senses non-methylated HSV CpG [213]. HSV DNAs and/or HSV-IC have been detected in the mouse cornea long after the infectious virus has been cleared [215,235,236]. When human corneal epithelial cells (HCE) and human corneal fibroblasts (HCRF) were infected with UV-inactivated HSV, transfected with HSV DNA or treated with HSV-IC, increased expression of TLR-3, -9 gene and IL-6 were observed in HCRFs [213]. Shortly after HSV-1 enters cells, virion shut off protein (vhs, UL41 gene) inhibits cellular RNA function [237,238]. However, IL-1 and IL-6 are still upregulated in the infected cells [239-241]. IL-6 induces expression of vascular endothelial growth factor (VEGF) and potentiates neovascularization [221,242]. Transfection of HCRF and a human macrophage cell line THP-1 [213] with HSV DNA or treatment with HSV-IC produces VEGF. Production of MMP-9 was elevated in

THP-1 cells but not in corneal cells [233]. THP-1 cells may produce MMP-9 via TLRs because THP-1 cells inhibited MMP-9 production with anti-TLR-2, -3 and -4 antibody treatment [213,243]. IL-1β, IL-6 and TNF-α also induce VEGF and MMP-9 expression in corneal cells and macrophages. Continued presence of HSV DNAs and HSV-ICs in the cornea may trigger inflammation and angiogenesis in HSK. VEGF and MMP-9 induction by IL-6 may be a therapeutic target via TLRs mediated cytokine pathways [222,244].

Dichotomy of innate immune responses in corneal herpes

In mouse model, new vessels sprout on the cornea after HSV-1 infection [245]. PMNs pass through these leaky vessels to the afflicted cornea [234]. PMNs are the prominent infiltrating cells in acute herpetic keratitis in patients and experimental animals [31,246]. Chemotactic factors, IL-8, Gro-α and granulocyte/macrophage colony-stimulating factor (GM-CSF) were released from corneal cells and macrophages infected with HSV-1 or treated with virus components. They attract and/or hold PMNs at the afflicted site, activates and prolongs survival of them [228,247]. PMNs cocultured with HCE, THP-1 cells or virus components produced highly reactive oxygen species (hROS) [228,247]. Such activated PMNs suppress HSV growth [228]. When they were overlaid onto the HSV-1-infected Vero cells, virus growth was inhibited [229]. Supernatants of the PMNs obtained after cocultured with HSV-infected or virus components treated HCE or THP-1 cells, HSV growth was halted [229]. TNF-α released from the PMNs was not enough for virus inhibition. IFN-α, -β and -γ were not released from PMNs mixed with treated HCE and/or THP-1 cells supernatants. H2O2 was released from PMNs and THP-1 cells infected with Mckrae, transfected with HSVDNA or treated with HSV-IC. H2O2 inhibited HSV-1 growth directly [229]. Myeloperoxidase (MPO) was also released by PMNs, contributing virus inhibition [229]. PMNs released NO in the supernatants when they were mixed with supernatants of HCE or THP-1 cells treated with HSVDNA and HSV-IC. Released NO was significantly higher concentration than those obtained from untreated HCE supernatant, however, it does not directly inhibit viral growth at the range of the concentrations obtained from treated HCE [229]. Low concentration of NO released from PMNs may convert naïve CD4 T cell to Th1 cells [248]. Neutrophils produce other factors like monokine induced by interferon γ (MIG). MIG induces and accumulates CD4+ Th1 cells [249]. When PMNs and CD4+ T cells were mixed and incubated with HCE supernatants treated with HSV components, MIG was released [229]. These local environments may contribute to the peripheral naïve CD4 +T cell differentiation to Th1 but further studies are required for this problem.

Prevention: Vaccine

Effective HSV vaccine has been tried including a nonvirulent virus, viral proteins, viral DNAs etc. [250-253]. However, none of them were effective in clinical trials. To obtain a better vaccine, delivery of effectors to the target is critical.

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