PERSPECTIVE

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Rapid host immune response and viral dynamics in herpes simplex virus-2 infection

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Herpes simplex virus-2 (HSV-2) is periodically shed throughout the human genital tract. Although a high viral load correlates with the development of genital ulcers, shedding also commonly occurs even when ulcers are absent, allowing for silent transmission during coitus and contributing to high seroprevalence of HSV-2 worldwide. Frequent viral reactivation occurs within ganglia despite diverse and complementary host and viral mechanisms that predispose toward latency, suggesting that viral replication may be constantly occurring in a small minority of neurons at these sites. Within genital mucosa, the *in vivo* expansion and clearance rates of HSV-2 are extremely rapid. Resident dendritic cells and memory HSV-2 specific T cells persist at prior sites of genital tract reactivation and, in conjunction with prompt innate recognition of infected cells, lead to rapid containment of infected cells. The fact that immune responses usually control viral replication in genital skin before lesions develop provides hope that enhancing such responses could lead to effective vaccines and immunotherapies.

HSV-2 causes a lifelong infection that is the leading cause of recurrent genital ulcers worldwide. In immunocompetent hosts, HSV-2 mucosal ulcerations are normally self-limited. However, systemic complications such as recurrent meningitis, hepatitis and pneumonitis can occur during acquisition or reactivation of infection, particularly among individuals with poor T cell immunity owing to AIDS, organ transplantation or chemotherapy[1–3]. Neonatal HSV infection arises from contact with the virus during childbirth and, when untreated, results in high mortality (>80%) and neurological morbidity[4–6].

HSV-2 infection is widespread and continues to spread efficiently across the globe. In addition to its high worldwide prevalence (**Fig. 1**), its recent incidence in several African cohorts approximates 20 infections per 100 person-years[7–10]. Global incidence is also estimated to be approximately 23.6 million infections per year[11]. The rate of HSV-2 coital acquisition as well as the serologic prevalence of HSV-2 is higher in women than in men[12,13], although men who have sex with men remain at high risk of incident infection[7]. Prevalent HSV-2 infection has been shown to increase the per-coital transmission rate of HIV five- to tenfold[14]. Men and women who have acquired HSV-2 have a two- to threefold increased risk of HIV-1 infection[15,16]. In countries in which sexually active adults have a high prevalence of HSV-2 infection, or in subpopulations such as men who have sex with men where HSV-2 infection is common, HSV-2 is a major epidemiological driver of HIV-1 epidemics[16–18].

Here we describe how frequent reactivation and release of HSV-2 from latency within neurons, as well as highly dynamic interactions between replicating HSV-2 and the host cell-mediated immune response in genital tissues, contribute to observed disease manifestations, high global prevalence and enhanced HIV acquisition risk. We also highlight the unique challenges that the kinetics of these viral-host interactions pose for antiviral and vaccine development. A crucial discovery over the last two decades is that HSV-2 is frequently shed throughout the human genital tract even when symptomatic ulcerations are not present. Although latency is the predominant state of the virus on a per-neuron basis within the entire biomass of the ganglia, HSV-2 is rarely quiescent, resulting in a frequent if not constant interplay between the virus, infected keratinocytes and the host immune cells in the genital tract.

Viral shedding and transmission of HSV-2

Many of the insights about mucosal HSV-2 infection have come from human studies using quantitative detection of HSV-2 by PCR-based techniques. Genital shedding is assessed quantitatively with genital swabs processed to detect HSV-2 DNA; this is widely accepted as a more sensitive, but no less specific, measure of shedding as compared to viral culture[19,20]. HSV DNA replication is the target of current antiviral therapies[19], and more severe disease manifestations correlate with higher shedding rates[21,22]. Whereas 80% of HSV-2–seroprevalent individuals show no genital lesions[23], the majority will have HSV-2 DNA commonly detected on genital swabs with repeated sampling. In a cohort of 531 asymptomatic and symptomatic HSV-2–seropositive individuals from the general population who were sampled daily over a 30- to 60-day time period, HSV-2 DNA was detected on 18% of days and occurred in the absence of recognizable genital lesions 67% of the time[24]. Both clinical and subclinical shedding episodes decrease only modestly over a 20-year time frame, highlighting the lifelong transmission potential of an infected person[25]. Efficient sexual transmission is therefore explained both by the large reservoir of persistently infected persons with undiagnosed infection and the high frequency of silent reactivation throughout the lifetime of the human host.

Viral shedding is characterized by frequent, highly heterogeneous bursts, or episodes. Recent studies indicate that subclinical shedding episodes can occur weekly, and often in multiple areas on the same

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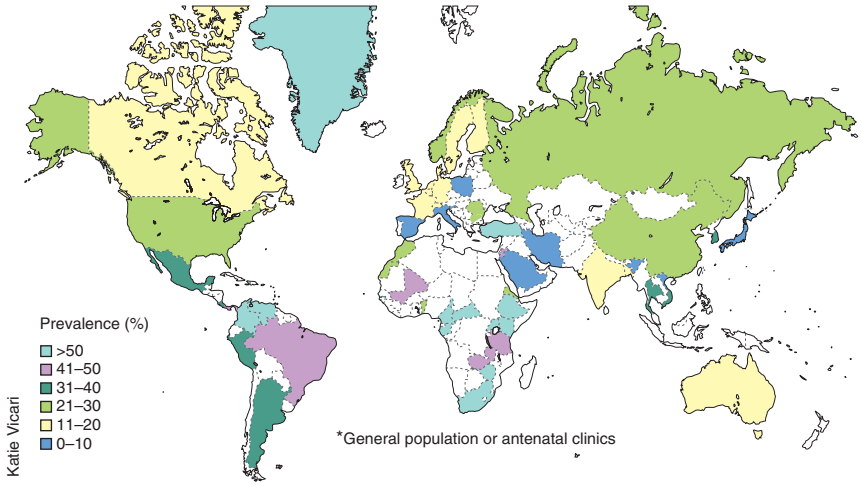

Figure 1 Prevalence of HSV-2 infection in women. Map is based on published studies within the last 15 years using serological assays that can accurately differentiate HSV-2 from HSV-1 infection. White indicates no available data.

day, indicating that reactivation occurs despite diverse and complementary host and viral mechanisms within ganglionic tissue that predispose toward latency. Shedding episodes also may vary enormously in duration and viral production over short time periods, owing to varying degrees of localized immune control within a person across space and time. Whereas replication and cell-to-cell transfer of HSV-2 are extremely rapid within genital lesions, resident memory HSV-2–specific T cells persist at the sites of reactivation; these highly localized infiltrates of dendritic cells (DCs) and $CD4^+$ and $CD8^+$ lymphocytes, in conjunction with prompt innate recognition of infected cells, often lead to rapid containment of infected cells and virus. Persistent $CD4^+$ T cells may be deleterious in that they enhance the probability of HIV-1 acquisition. In contrast, the fact that cell-mediated immune responses control virus replication in genital skin before the development of genital lesions provides optimism that enhancing such responses will lead to the design and development of immunotherapeutic approaches that control subclinical reactivations and to effective vaccines for the prevention of HSV-2 acquisition.

Infection is acquired almost invariably via sexual contact with infected genital secretions and as such is decreased by condom use[26]. The relationship between viral reactivation rate and titer and viral transmission is unknown. A retrospective study estimated a median of 40 sexual acts[27], or ~3.5% per-coital transmission risk, before transmission. Whereas genital shedding episodes associated with visible lesions are of longer duration and account for the highest genital viral loads, epidemiologic studies indicate that the majority of transmissions occur during asymptomatic shedding of virus (**Box 1**)[28,29]. This may be explained by the fact that couples are probably more likely to avoid coitus while severe lesions are apparent. Moreover, many asymptomatic episodes, although associated with lower peak viral loads and duration compared to recurrences, are still associated with viral loads exceeding 10^6 HSV DNA copies and duration exceeding 3 days[24].

Antiviral therapy and vaccines. Current antiviral therapies limit the severity and frequency of genital lesions, but because they do not entirely eliminate episodes of subclinical shedding they only partially reduce transmission of HSV-2 to new partners. Treatment for mucosal HSV-2 infection has been available for over 30 years. HSV-2 was the prototype for development of small-molecule inhibitors of virally encoded replication enzymes. Three decades ago, the nucleoside analog aciclovir (ACV) was shown to be a substrate for HSV-1 and HSV-2 thymidine kinase[30]. Viral thymidine kinase selectively phosphorylates ACV and cellular enzymes add two additional phosphates, resulting in ACV-triphosphate, which is a chain terminator of HSV DNA synthesis. The elucidation of the mechanisms of action of ACV and its subsequent derivatives as clinically effective and safe drugs provided the conceptual framework for the development of antiviral agents for HIV, cytomegalovirus and hepatitis B and C.

ACV, its derivative valaciclovir (VCV) and the related nucleoside analog famciclovir (FCV) effectively decrease the severity of genital lesions in immunocompetent and immunosuppressed patients if given early during recurrences. In addition, twice-daily administration has low toxicity and decreases lesion rates by 75% and shedding rates by 80% (ref. 31). VCV, an ACV prodrug, achieves higher peak serum concentrations than ACV[32], and FCV has a considerably longer intracellular half-life than ACV and VCV[33]. However, recent studies indicate that subclinical breakthrough reactivations, especially of short duration, are common even among fully compliant study participants receiving maximal daily doses of VCV or FCV therapy[34], a phenomenon that may be explained by the rapid kinetics of viral expansion during drug trough levels. VCV was also the first antiviral to reduce transmission of a sexually transmitted viral infection (by ~50% among serodiscordant couples)[35]. Despite increasing use of these antivirals over the last 25 years, most infected persons remain untreated. Resistance to current anti–HSV-2 antivirals is infrequent and is usually encountered only among severely immunocompromised hosts[36]. This characteristic is relatively unique among antiviral agents.

To date, no effective human vaccine against HSV-2 exists. Attempts at using recombinant glycoprotein D, an envelope protein that generates a substantial neutralizing antibody response, have recently been shown to be unsuccessful[37]. A better understanding of the immunology of acquisition of HSV-2 is needed to successfully develop a vaccine that will reduce coital acquisition[38].

Pathogenesis of primary infection and latency in ganglia

HSV-2 accesses permissive nucleated cells in the mid- to basal epidermis via microscopic breaches in the epidermis that occur with coitus. The HSV replication cycle is governed by processes that promote transcription of viral DNA, translation of mRNA, DNA replication, viral assembly and viral egress before cell lysis[39,40], along with numerous immune evasion strategies at each stage of replication[41]. Viral replication within keratinocytes leads inevitably to cell death. After entry into the cytosol, viral protein production occurs in a cascade-like fashion. First, immediate-early gene products are produced within hours to promote immune evasion, neurovirulence and synthesis of early proteins required for viral DNA replication. Late proteins then provide structural components of the capsid, which are necessary for viral egress. The timeline between viral entry and cell lysis is approximately 24 hours[40]. In the absence of antiviral therapy, HSV spreads rapidly between epithelial cells, and subsequent cell lysis

BOX 1 Glossary of terms

Asymptomatic shedding: The detection of HSV-2 DNA or the isolation of HSV in tissue culture from the genital or rectal mucosa at times when no characteristic signs of clinical genital herpes are present; sometimes called subclinical infection.

Clinical herpes: The presence of genital ulcerations, fissures and/or vesicles, usually in association with pain, itching, and/or dysuria. The symptoms may vary with the location of the lesion and are also called 'symptomatic episodes'.

Mucosal shedding: Detection of HSV-2 on a mucosal surface by either culture or DNA PCR, resulting from HSV-2 release from neurons followed by replication in the genital epithelium.

Recurrences or symptomatic episodes: Any instance of clinical herpes after the first episode following virus acquisition. An episode of clinically recognized herpes usually lasts from 1 to 7 days and is often associated with multiple days in which HSV is isolated from mucosal or skin swabs or samples.

Viral latency: Any time after acquisition during which the virus remains in a dormant, reversible nonreplicative state in the ganglia.

Viral leak: Release of virus down axons from neuronal cell bodies within the ganglia to the dermal-epidermal junction in the genital skin or mucosa; the antecedent process that results in subsequent infection of adjacent epithelial cells.

Viral reactivation: HSV-2 DNA replication and production of virions within ganglia, representing a break from viral latency.

leads to vesicle or ulcer formation. The virus enters sensory neuronal axons via a plexus of free nerve endings in the epidermis and is transported into neuronal cell bodies in the dorsal root ganglia, where it replicates and spreads to other neurons. Little is known about the extent of replication and factors controlling neuronal infection and spread during HSV-2 acquisition.

Latency and reactivation. The viral genome is maintained within ganglia for the life of a host in a quiescent condition termed latency (**Box 1**). Reactivation within ganglia leads to transport of HSV-2 toward genital skin or mucosa, and subsequent replication within epidermal cells after passage of viruses across the axonal-epithelial gap results in discrete shedding episodes, which are commonly subclinical[42]. Several lines of evidence suggest that detection of asymptomatic HSV-2 DNA with a genital swab denotes viral replication within epithelial cells rather than simply either viral release from neurons or micro-trauma from swabbing: first, the plexus of free nerve endings arborizes and terminates in the stratum granulosum of the mid-epidermis (**Fig. 2**), and a minimum of ten epithelial cells separates neuron endings from the genital skin surface, indicating that the architecture of this layer of epithelial cells must be substantially disrupted for swabs to contact the mid-epidermis; second, per-cell amplification of HSV DNA is several logs higher in cultured epithelial cells than in neurons;[43,44] and finally, aciclovir therapy, which interacts with viral genes made only during the replication cycle of the virus, reduces the frequency and titer of such shedding, though this could be the result of activity in neurons as well as keratinocytes.

Symptomatic episodes, characterized by closely arrayed visible ulcers and vesicles on a bed of erythema, are termed recurrences (**Box 1**). Mathematical estimates derived from examining cell size and the histologic structure of genital mucosa suggest that a single visible ulcer of at least 1 mm in diameter implies the death of >14,000 epithelial cells[45]. Recurrences occur infrequently in most persons (0–10 times yearly). This observation led to the view that latency was comprehensively controlled within all neurons in sacral ganglia requiring infrequent 'trauma', environmental stimuli such as ultraviolet light exposure or immune suppression (transient or sustained) to elicit reactivation.

In the last decade, several experimental and clinical studies have altered the concept that viral reactivation is infrequent and usually results in genital lesions. Recent data support the concept that a minority of viral genomes in ganglia may be reactivating at any given point in time. The frequent detection of subclinical episodes of mucosal shedding in over 90% of persons who possess HSV-2–specific antibodies undermines the idea that viral release from ganglia is uncommon and occurs with the same frequency as recurrences[19,45–49], and it may suggest that neuronal stimulation leading to viral release

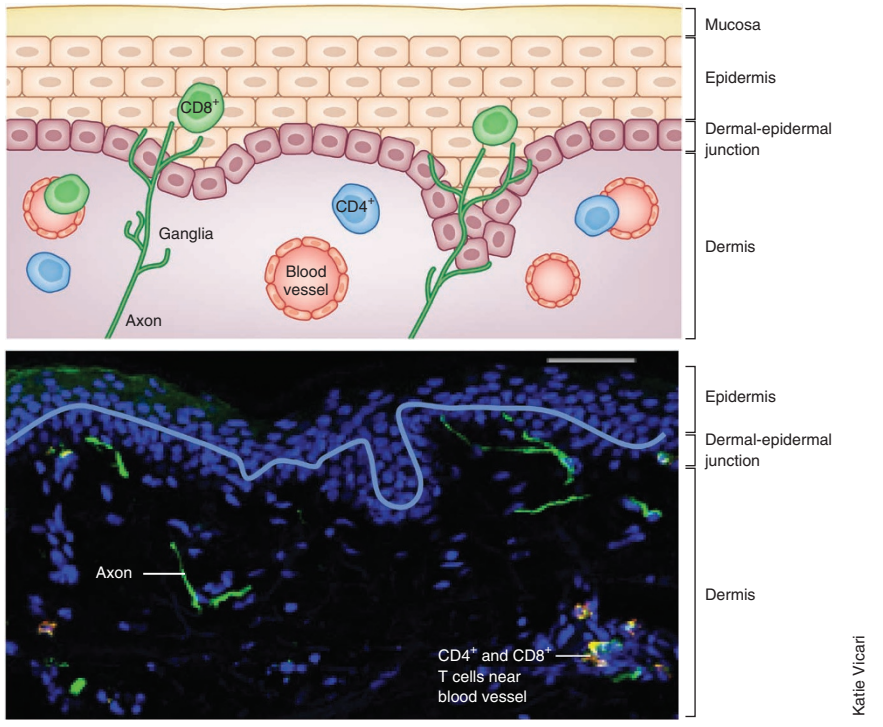

Figure 2 Schematic and histologic representation of healthy genital mucosa. Axons arrive from the dorsal root ganglia and terminate at the dermal-epidermal junction or within the mid-layer of the epidermis. HSV-2 is released from axonal termini across the neuron-epithelial gap and can enter epithelial cells where virus is amplified and rapidly spreads to contiguous skin cells. Low numbers of $CD4^+$ and $CD8^+$ T cells are present in the dermis and dermal-epidermal junction after entering the genital skin through blood vessels. The white line denotes the dermal-epidermal junction. Scale bar, 50 μm.

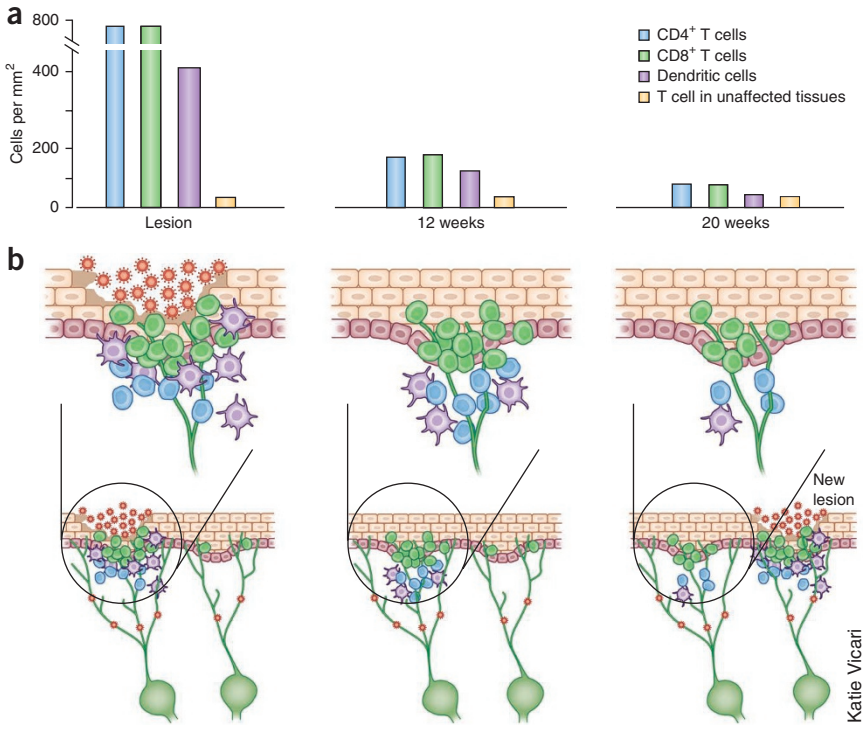

Figure 3 HSV-2 reactivation dynamics. HSV is continually and slowly released due to concurrent reactivation of HSV within multiple ganglia. This leads to HSV release into multiple spatially discrete mucosal sites. Periodically, epithelial cells become infected, leading to rapid viral spread, multiple infected cells, microulcer or ulcer formation (where high numbers of keratinocytes are lysed and virions are shed) and dense CD4+ and CD8+ T cell infiltration. After containment of HSV at a single site, T cells persist at that location for several months in an immunosurveillance capacity. HSV-2 reactivates at other sites where CD4+ and CD8+ lymphocyte density is lower, allowing for a genital tract shedding episode, which initiates in a different region. (**a**) Lymphocytes and DCs remain localized at the lesion site for months after healing and decrease in numbers over time. (**b**) HSV-2 (red-orange) drips from neurons (long green cells) and periodically will infect epithelial cells (burgundy), resulting in lesion formation, where high densities of CD4+ (blue) and CD8+ (green) lymphocytes, and to a lesser extent DCs (purple), traffic to clear viral infection (left side of mucosa in left drawing); levels of these immune cells gradually decay over time (left side of mucosa in right drawing); sites only millimeters away from HSV-2 replication can remain uninfected, and T cell density in these regions remains low (right side of mucosa in left panel) rendering these sites susceptible to high-titer shedding episodes in the ensuing weeks (right side of mucosa in right drawing).

is a common event. Detailed studies in which HSV-2–infected individuals were sampled at 6 h intervals for at least 1 month revealed that shedding episodes occur roughly every 10 days and that most subclinical reactivations are rapidly cleared in 2 to 12 hours[46]. HSV-2–specific lymphocytes persist in genital skin contiguous to the neuronal termini of sensory nerve roots[50], which suggests an active role in immune surveillance perhaps due to frequent contact with released HSV.

Because direct observation of viral release from neural root ganglia toward the skin is not possible in humans owing to lack of access to such tissue, one of the unknowns in HSV-2 pathogenesis is the frequency and pattern of viral release or leak from neurons. Although available cell culture systems[43], and guinea pig models[51–53], can capture the passage of HSV from neuronal cell bodies to epithelial monolayers, they lack sufficient semblance to recapitulate the full complexity of viral dynamics and the immunologic response in human infection. Statistical analysis of HSV-2 shedding patterns derived from sampling a wide anatomic area with genital swabs suggests that many episodes involve multiple concurrent ganglionic reactivations and that not all virus released from neuronal endings in the genital tract necessarily leads to detectable shedding episodes[47]. A mathematical model of HSV-2 reactivation, that assumed the total body of dorsal root ganglia releases small amounts of virus in a nearly constant fashion (10–100 HSV-2 DNA copies per day), most closely fit available shedding data sets[45]. This concept of 'slow viral drip' is in concert with the observation that 6.3% of neuronal cell bodies in human trigeminal ganglia harbor HSV-1 DNA, whereas only 0.6% contain >100 HSV-1 DNA copies[54]. Many infected neuronal cell bodies are surrounded by host T cells. These calculations help harmonize the idea of a nearly constant 'leak' of HSV at the whole-organism level with the well-known concept that the overwhelming majority of viral DNA within neurons is in a latent form. It is the wide anatomic distribution of these reactivating virions that seems to result in frequent antigenic exposure to the host across many micro-regions in the genital tract (**Fig. 3**).

Latency is defined at the cellular level as the presence of fully competent viral genomes in cell nuclei without production of infectious virus (**Box 1**). Although recent evidence suggests the presence of latent genomes in autonomic tissues[55], as well as within sensory neuron cell bodies, there is no evidence to date that nonreplicating viral DNA can persist within keratinocytes or other non-neural tissues. For this reason, we believe that detection of a newly initiated HSV-2 DNA shedding episode in the genital tract implies recent HSV-2 replication in, and transport from, neuronal ganglia. Neuronal latency is interrupted when translation of a full complement of viral lytic proteins occurs (**Fig. 4**)[56]. This is followed by HSV glycoprotein E- and I-dependent anterograde transport of viral capsids and glycoproteins down axons toward the genital tract[44,57]. The architecture and function of ganglia are preserved without apparent cell loss. Accordingly, patients with genital herpes do not experience genital dysesthesia between recurrences even after decades of frequent reactivation[42].

Molecular features of latency. Factors that determine the balance between latency and reactivation within a single neuron are not completely understood. In the 1980s, Stevens *et al.*[58] defined a series of latency-associated transcripts (LATs) in neuronal cell bodies within trigeminal and sacral ganglia. Although the exact role of LATs in maintaining latency is still debated, viral genomes are found in cells expressing and not expressing LATs, suggesting that viruses within different neurons may be in distinct stages of latency and reactivation[54,59]. Even in mouse ganglia, where spontaneous reactivation does not occur, abundant expression of proteins from all stages of HSV replication is evident in a small percentage of trigeminal neuronal cell bodies[60], which are targets for an HSV-directed inflammatory infiltrate[61,62].

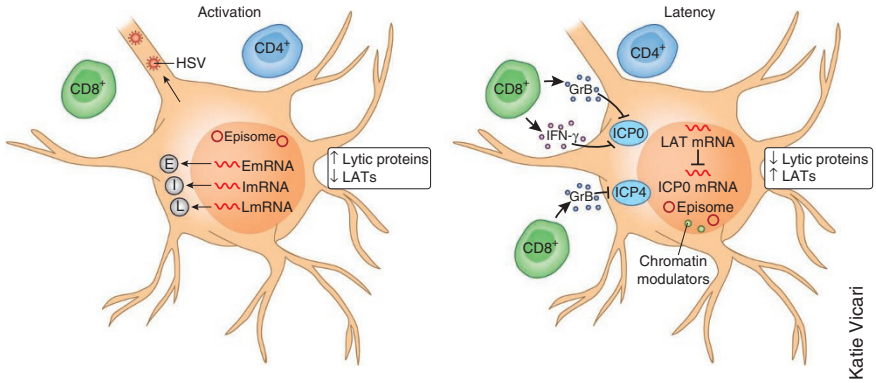

Figure 4 HSV-2 and host interactions in nerve root ganglia. Depiction of the molecular interactions in the ganglia showing the inverse relationship between LATs and lytic proteins. Activation comprises active transcription of viral RNA and high lytic protein expression accompanied by low LAT production. Latency comprises control of HSV protein expression in neuronal cell bodies in the ganglion by local resident CD8+ T cells: IFN-γ and granzyme B (GrB) released by host T cells may inhibit lytic protein expression. LAT production is high, and lytic protein production is low. ImRNA, immediate early RNA; EmRNA, early RNA; LmRNA, late RNA; ICP, infected-cell polypeptide.

LATs block HSV-1–induced neuronal apoptosis in animal models[63] and may also protect neurons from CD8+ T cell– and granzyme-induced apoptosis[64]. HSV-2 expresses two 22-nucleotide miRNAs (part of the LAT) in human dorsal root ganglia that are antisense to ICP0, an immediate-early protein necessary for lytic replication (**Fig. 4**)[65]. These miRNAs perform post-transcriptional regulation that could potentially be involved in maintenance of latency[66]. Undefined cell-type–specific factors also cause HSV to preferentially express gene products that modulate host chromatin structures, which in turn favors downregulation of immediate-early gene products. This contrasts with epidermal cells where an alternative chromatin structure favors lytic gene expression[67].

Host immunity in ganglia. Whereas the ganglia were once considered immunoprivileged sites where HSV could exist with low antigenic presence and transcription of few available viral genes[58], there is now evidence that host immune mechanisms may favor latency. HSV-1–specific effector memory CD8+ and CD4+ T cells are permanently retained within trigeminal ganglia in infected humans[68], though it is unknown whether similar cell subsets exist within the dorsal root ganglia during HSV-2 infection. Histologic evidence is consistent with a noncytocidal mechanism controlling levels of virus in neurons. As has been demonstrated for hepatitis B infection, CD8+ T cells are known to maintain control over virus-infected cells using noncytocidal as well as directly lytic mechanisms[69]. Mice have a similar localization of lymphocytes in trigeminal ganglia, and CD8+ T cells prevent spontaneous reactivations even though viral proteins are present in miniscule amounts and neurons do not express major histocompatibility complex molecules necessary for T cell binding[61]. This mechanism of memory effector function results from CD4+ T cell help early during infection. Memory CD8+ T cells produce interferon-γ (IFN-γ), which has a partial dampening effect on early viral replication within neurons, and disruption of these cells promotes reactivation[70,71]. CD8+ T cells may polarize their T cell receptors toward infected neurons and release granzyme B lytic granules (**Fig. 4**). Granzyme B induces apoptotic pathways in fibroblasts, but not in neurons where it cleaves ICP4, a viral protein needed for transcription of subsequent essential early and late HSV genes. Early viral replication may therefore be limited, ultimately favoring viral latency without lethal effects to the neuron[72].

Our opinion is that HSV-2 latency is the overwhelmingly predominate state on a per-neuron basis, but that HSV-2 is rarely quiescent within the entire biomass of ganglia and that there may be an evolving interplay between virus, neurons and acquired immune cells[73]. The idea that reactivation is a common, if not constant, phenomenon, perhaps induced by repeated neuronal stimulation, does not obscure the role that ganglionic immune responses might have in disease phenotype. In mice, the number of infected neurons and average genomic copies per neuron correlate with total ganglionic latent viral load[74]. Ganglionic viral load is determined in part by inoculums during primary infection[75], remains stable during chronic infection[76], and in guinea pigs and mice predicts reactivation rate[75,77], which is in turn inversely correlated with ganglionic CD8+ T cell density[75]. *In silico* models suggest that even small adjustments in the average amount of HSV-2 released from neurons per day may have important effects on shedding frequency[45]. Some heterogeneity of reactivation frequency in humans might be due to variability of host immune control in neural ganglia.

Transition from neuronal to mucosal infection

A key mystery in HSV biology is the fate of virions released from neurons into genital skin. One unique feature of infection is extreme variability between genital reactivations. The same individual over a span of 1–2 weeks may show several subclinical episodes lasting only 2–12 hours, followed by a lesional episode lasting 4–10 days[46]. Viral production can differ by a multiple of 10 million between successive episodes[19,46,48]. There is a direct correlation between quantity of genomic HSV-2 detected, episode duration and the presence of visible genital lesions. Yet all mucosal reactivations in the immunocompetent host are notable for extraordinarily rapid expansion (minimum 7.6 $\log_{10}$ HSV-2 DNA copies per day) and decay (minimum 6.2 $\log_{10}$ HSV-2 DNA copies per day) phases[24]. Any comprehensive theory of HSV-2 pathogenesis must explain the profound heterogeneity in episode severity in the same individual over time, as well as the rapid production and elimination of HSV-2 in the genital tract.

A reliable animal model system for HSV-2 reactivation would be of enormous utility for the field. Spontaneous HSV-2 reactivation is rare in mice unless the ganglion is 'stressed' or the mouse is immunosuppressed, at which time relatively efficient release of virions and expansion of infection into the skin and mucosa occurs. Sporadic reactivation occurs in guinea pigs after resolution of primary infection[78], but this model has not been evaluated in enough detail using molecular detection of HSV to understand whether the kinetics of reactivation and clearance approximate those of human infection. Guinea pigs can be effectively vaccinated against HSV-2 acquisition with glycoprotein D subunits[79], and an immunotherapeutic benefit is achieved when lower doses of vaccine are used[80]. The lack of an effective glycoprotein-based vaccine in humans highlights the fact that certain key features of human and rodent viral immunity probably differ in important ways.

Antiviral innate immunity in the genital mucosa. Mucosal shedding episodes are probably initiated after successful attachment

of neuron-derived HSV into at least one epithelial cell. The immune response is initiated soon thereafter. HSV recognition occurs even before entry and replication via several pathways, including recognition of essential viral fusion proteins (gH and gL)[81], and genetic deficits in innate immunity can be associated with severe infections. The HSV immediate-early protein ICP27 is required for optimal nuclear factor-κB (NF-κB) expression within 3–5 hours of infection[82]. Mutations in NEMO/IKK-γ, an NF-κB regulator, are associated with fatal HSV-1 infection[83]. Autosomal-dominant Toll-like receptor 3 (TLR3) deficiency permits high-level viral replication in fibroblasts due to abnormal IFN-β and IFN-γ production, resulting in an increased risk of fatal HSV-1 encephalitis despite normal resistance to severe forms of other viral infections[84]. TIR-domain-containing adaptor-inducing interferon-β (TRIF) deficiency impairs TLR3 and represents another genetic marker for increased risk of sporadic childhood HSV-1 encephalitis[85]. TLR2 on the surface of DCs and TLR9 within their endosomes detect HSV pathogen-associated molecular patterns in quick succession, also activating NF-κB pathways[86]. Plasmacytoid DCs (pDCs) subsequently exert a strong antiviral effect by secreting type I IFNs[87]. Consanguineous family members who develop fatal HSV encephalitis seem to have a poor IFN-α response to TLR7 and TLR9 agonists as a result of deficits in TLR signaling within endoplasmic reticula[88]. The genetic link between innate immune deficiencies and HSV disease severity, particularly risk for early encephalitis, has been reviewed elsewhere[89].

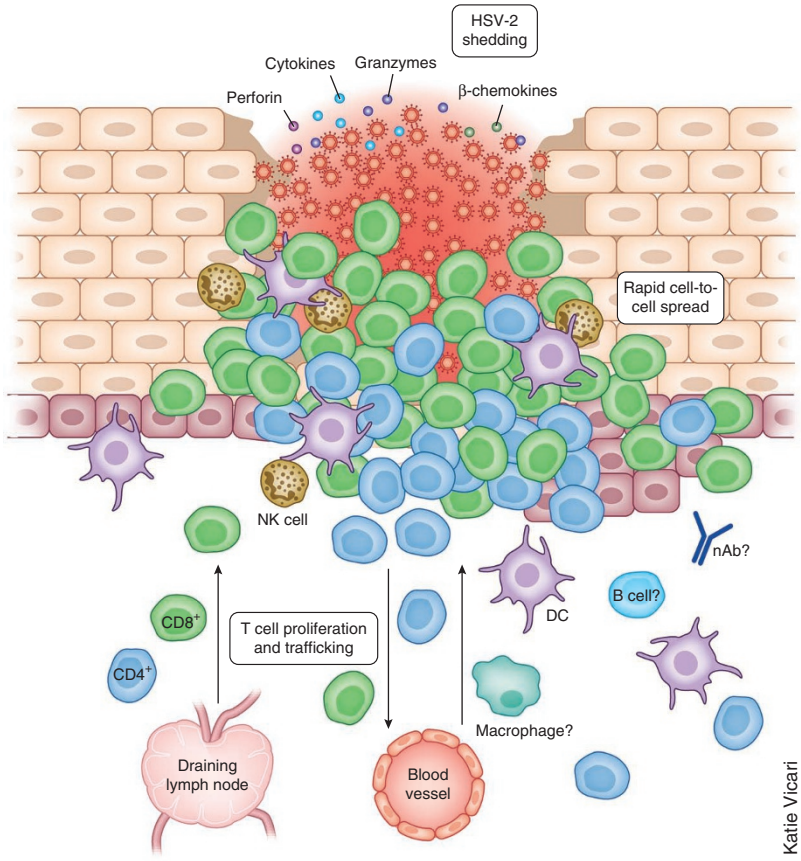

Figure 5 Host immunity in the genital mucosa. There is an intense infiltrate of CD4+ (blue) and CD8+ (orange) T cells and mixed populations of DCs (purple), macrophages (teal) and NK cells (olive). This cellular infiltrate produces IFN-γ, tumor necrosis factor-α (TNF-α), RANTES, granzyme B and perforin. B cells (aqua cells) and neutralizing antibodies (nAb) may also be present. The location and function of macrophages, B cells and neutralizing antibodies have not been fully characterized.

Herpetic vesicles are packed with high amounts of cytokines, β chemokines[90] and IFNs[91], and early cytokine production of epithelial origin is geared toward recruitment and activation of T cells[90]. IFN-β is produced by keratinocytes and has a potent antiviral effect during HSV infection of these skin cells[92], whereas IFN-α and IFN-γ seem to have inhibitory effects on viral spread both from neurons to epithelial cells and between epithelial cells[93]. Several immediate-early viral proteins interrupt activity of type I IFNs[41], and HSV-2 seems to block IFN-α and IFN-β production early during recurrences in humans[94]. Type II IFNs are secreted by antigen-specific CD8+ and CD4+ T cells and innate lymphocytes such as nature killer (NK) cells and γδ T cells, which are all present in genital lesions. IFN-γ binds different receptors than type I IFNs but triggers a similar transcriptional response involving signal transducer and activator of transcription-1 (STAT-1) and STAT-2; STAT-1 mutations are associated with fatal HSV-1 infections[95].

The high frequency of shedding episodes in the genital tract suggests that viral evasion mechanisms frequently at least partially overcome innate responses. This may be due to frequent physiologic stresses that temporarily suppress local immunity. Ultraviolet light (UV) is one such stress that seems to hamper viral antigen presentation[96] and cause photoisomerization of *trans*-urocanic acid to *cis*-urocanic acid, which in turn induces upregulation of immunomodulatory factors in keratinocytes[97]. This may be one explanation for the observation that UV exposure after HSV inoculation in rats leads to far more severe lesions despite an apparent lack of effect on cell-mediated immune response[98]. Whereas the relevance of UV exposure to human orolabial HSV-1 infection is clear, the particular stresses that might predispose to genital HSV-2 reactivation are unknown. Nevertheless, because innate mechanisms are operative early during viral-host interaction, interventions that stimulate innate responses to viral antigen hold promise. For example, a TLR7 and TLR8 agonist that induced type I IFNs when applied topically to human genital skin was associated with decreased HSV-2 shedding frequency[99].

Induction of acquired mucosal immunity. In 1985, Cunningham *et al.*[100] discovered that intense inflammation develops in genital lesions and comprises a wide variety of cells (**Fig. 5**), including a predominance of T cells and lesser concentrations of B cells and NK cells in the first 24 hours, followed by monocyte and macrophage infiltrates in the later stages of healing. Innate signaling and acquired immune responses are linked via DCs, NK cells and cytokine signaling pathways. pDCs are typically restricted to blood and lymphatic compartments but infiltrate the dermal-epidermal junction and interface with infected cells, activated T cells and NK cells during lesions. In culture, HSV-exposed pDCs are not permissive for viral replication but do stimulate autologous T lymphocyte proliferation[101]. Activated pDCs

potently induce cutaneous lymphocyte antigen (CLA) on HSV-2–reactive CD4+ T cells, probably through the release of cytokines[102], which subsequently promotes T cell trafficking to lesion sites[103]. pDCs also boost adaptive immune responses in draining lymph nodes[104]. pDC-depleted mice poorly control HSV after intravaginal challenge[87], and severe human infections may result from defective pDCs[105,106]. Thus, trafficking of pDCs to the dermal-epidermal junction may play an important part in containment through the recruitment of effector lymphocytes to the site of infection.

Monocytes are recruited to inflamed skin sites and form monocyte-derived DCs (MDDCs), which locally cross-present viral antigen to memory and effector T cells[107]. In mice, HSV-infected MDDCs undergo apoptosis and are phagocytosed by uninfected MDDCs[108]. This occurs in draining lymph nodes, where viral DNA is not detectable by PCR, but antigen presentation results in HSV-specific T cell activation within 6 hours and proliferation within 30 hours[109]. Langerhans cells, the most prominent antigen-presenting cells in skin, surprisingly do not prime T helper type 1 cells[110], whereas other migratory DC subsets, which first recognize viral presence in skin, are crucial in this role[111]. Among migratory DCs, the CD103+ subset seems specifically adapted for cross-presentation to naive CD8+ T cells, whereas CD11b+ dermal DCs successfully present to CD4+ lymphocytes. CD103+ DCs have a dominant role in presenting antigen to CD8+ T cells in mouse skin after secondary infection as well[112]. Whereas Langerhans cells seem to be the first DCs to emigrate from skin after infection, CD103+ cell migration from infected skin appears to predominate more than 24 hours after viral inoculation[113]. The redundancy within the antigen-presentation system underscores the importance of rapid pathogen recognition and lymphocyte activation[114,115].

Antiviral antibody response. Several pieces of evidence suggest that humoral immunity may be important in controlling HSV infection. Neutralizing antibodies, which can bind viral envelope glycoproteins necessary for viral entry, develop during infection[116]. The presence of maternal antibodies specific to HSV-2 but not HSV-1 reduces neonatal transmission of HSV-2 (ref. 117). Antibodies limit the extent of mucosal and dorsal root ganglia infection, as well as recurrence rates in the guinea pig model, provided that antibody is delivered in high quantities during the first 24 hours of infection[118]. Neutralizing antibodies are capable of binding virus in the gap between neuron endings and epithelial cells and may limit bidirectional viral transfer between these tissues[119]. IgG antibody subsets predominate in the female genital tract, and Fc receptors on epithelial cells are required for transcytosis of IgG across these cells and for protection against HSV-2 challenge[120]. IgG seems to be a necessary component of the protective immune response in a humanized mouse model[121], and vaccine studies in guinea pigs using glycoprotein subunit products continue to show promising results, though induction of a robust CD4+ T cell response may explain this phenomenon[122].

In contrast, human vaccine studies using glycoprotein product have shown no protection against HSV-2 and only limited protection against HSV-1, despite generation of neutralizing antibody titers similar to those observed in natural infections[123,124]. Whereas T cell immunodeficiency is a clear risk factor for severe infection, individuals with agammaglobulinemia seem to be at no higher risk of severe infection than normal individuals. HSV-1 encodes complement-interacting and IgG Fc–binding glycoproteins (gC and gE) that may limit the *in vivo* effects of antibody-dependent cell-mediated cytotoxicity[125,126]. In addition, the effect of mucosal antibodies may be limited, as spread of infection within an ulcer occurs via direct cell-to-cell spread of virus across tight junctions that are inaccessible to antibodies[127]. For these reasons, the overall role of neutralizing antibodies in protection is unknown.

Mucosal T cell immune response. Substantial evidence in both humans and mouse models of HSV supports a crucial role for highly localized T cell responses in viral containment[128,129]. In mouse models, T helper type 1–mediated protection occurs via major histocompatibility complex class II (DC and B cell)-initiated CD4+ memory T cell re-stimulation within vaginal mucosa. Activated CD4+ cells produce IFN-γ, which limits viral production and spread[130]. Yet infection of mouse flank results in a rapid influx of a highly localized, nonmigratory subset of HSV-specific CD8+ memory cells that remain at the inoculation site for at least 100 days. These cells show lower homeostatic replication and greater expression of E-cadherin and extracellular matrix–binding proteins than circulating HSV-specific CD8+ lymphocytes. Their memory effector phenotype provides local protection against viral re-inoculation several months after initial exposure, whereas infection at a separate skin locus is poorly controlled[129]. Transplantation of CD8+ memory cells before HSV-1 inoculation in a mouse does not prevent establishment of latency but substantially limits accumulation of virus and infected cells in the skin and the innervating dorsal root ganglia[131]. Chronic re-exposure to HSV antigen does not seem to induce T cell exhaustion[132].

Similarly, functionally competent HSV-2–specific cytotoxic CD8+ T cells and CD4+ T cells densely populate the site of genital recurrences in humans (**Fig. 5**)[133–135]. CD8+ T cells selectively infiltrate locations adjacent to HSV-2–infected cells during lesions with no excess CD8+ T cell density even two centimeters away from the active lesion[50], which illustrates the important spatial dynamics between the virus and host. HSV-2–specific CD8+ lymphocytes also persist at the dermal-epidermal junction close to peripheral nerve endings (**Fig. 3**). Recent studies in which these CD8+ T cells are captured by laser dissection microscopy and then evaluated for transcriptional changes have shown that perforins, granzymes A and B, and a variety of antiviral cytokines are expressed during clinical quiescence, signifying an active immunosurveillance role after episode clearance[136].

CD4+ T cell infiltration occurs slightly earlier during lesion formation than CD8+ T cell infiltration[135]. DCs and CD4+ lymphocytes occupy a deeper location than CD8+ T cells, congregating in the perivascular space in post-capillary venule tufts in rete ridges of the upper dermis. CD4+ T cells also persist locally for at least 6 months whether or not patients receive antiviral therapy (**Figs. 3**) and, along with NK cells, continue to express IFN-γ early after exposure to HSV antigen after lesion healing[128]. IFN-γ tissue abundance and IFN gene product expression levels remain elevated in tissue for weeks after lesion healing, though it is not known if this cytokine is produced by macrophages or lymphocyte subsets[94]. The dense lymphocyte infiltration during lesions and persistence afterward has relevance to HIV-1 co-infection. CD4+ lymphocytes in genital skin are enriched for chemokine receptor CCR5, and CCR5-tropic strains of HIV-1 replicate in higher numbers in cells derived from healed lesion areas than from control skin, providing a biological mechanism for enhanced HIV-1 acquisition in HSV-2–infected individuals[128].

An unanswered question in human infection is whether lymphocyte replenishment at the site of an HSV-2 reactivation is due to trafficking from secondary immunologic organs or local expansion. For circulating CD8+ cytotoxic lymphocytes to enter infected tissue, CD4+ T cells must first encounter antigen presented by DCs in the submucosa[107] and then produce interferon-γ. IFN-γ–induced chemokine

production by epithelial cells allows for cytotoxic lymphocyte homing to locations where viral antigen is present on infected cell surfaces[137]. Plasma central memory CD4+ and CD8+ T cells specific for HSV-2 constitutively express CLA, whereas lymphocytes specific for non–skin-associated herpes viruses lack this antigen: CLA promotes chemotaxis by adhering to upregulated E-selectin on HSV-2–infected epithelial cells[102,138]. HSV-specific CD8+ T cell precursors are recruited into the cytotoxic T lymphocyte response within 24 hours of mouse footpad inoculation[139]. The potential importance of lymphocyte trafficking is demonstrated by the fact that lower levels of HSV-specific CD8+ cytotoxic T lymphocytes among peripheral blood mononuclear cells in HIV-infected individuals predict more severe recurrences[140].

This multistep process of viral antigen transport from site of infection to secondary lymphoid compartments, antigen presentation to cognate CD8+ T cells, lymphocyte activation and expansion within secondary lymphatic tissues, and migration back to the site of reactivation occurs while the number of HSV-2–infected cells rapidly expands. Greater control might be attained if mucosal effector cells persisted for a more extensive time period. In mouse models, the immunodominant structural protein gB is presented on the surface of infected cells with the kinetics of an immediate-early protein, allowing for CD8+ T cell activation within 2 hours[141]; local CD8+ re-expansion from local DC and CD4+ T cell priming may therefore be a key component in controlling localized infection[107].

More general investigations of viral immunity, such as that of murine cytomegalovirus, suggest that proliferating, terminally differentiated effector memory T cells persist at the point of viral infection[142]. Following certain viral infections of the skin and gut, non-recirculating memory CD8+ T cells that have replicative and cytolytic function remain at the infection site, allowing for immediate recognition and clearance of pathogens at the portal of entry[143]. The ability of fully competent effector memory T cells to traffic to the periphery and then persist in previously infected tissues, while maintaining an ability to rapidly recognize and eliminate infected cells, is therefore not likely to be unique to HSV infection[144], though HSV-2 infection provides a unique opportunity to observe these phenomena in the human host.

Temporospatial fluctuations in densities of localized CD8+ T cells, CD4+ T cells, DCs, macrophages, NK cells and IFN-γ[50,94,128] may explain the enormous diversity of shedding episode severity witnessed in the same infected person over time. Replication and spread of virus is explosive during some reactivations, leading to thousands of infected cells within 24 hours. In support of this finding, surface glycoprotein gE can mediate cell-to-cell spread at keratinocyte tight junctions, facilitating efficient and rapid infection of tightly packed epithelial cells[145]. Yet the host controls most reactivations within several hours before formation of genital lesions occurs. Differences of only 20–30 minutes in the average lifespan of a virally infected cell seem to determine whether virus is contained as a subclinical shedding episode lasting <24 hours rather than a 4- to 8-day clinical recurrence[146]. Thus, the spatial interactions between viral spread and containment can explain the varying heterogeneity of episodes within a single immunocompetent individual.

The margin between episode containment within the first hours of viral replication and progression to a prolonged episode seems extremely narrow and may pertain to the lack of a localized host immune response at small clusters of productively infected epidermal cells. A possible complementary explanation involves the immunomodulatory effects that HSV has on activated T cells. During lesion formation, HSV infects 5–20% of skin-resident CD4+ and CD8+ lymphocytes via a virologic synapse from epidermal cells[147]. Activated T cells do not support viral replication, but HSV alters lymphocyte receptor signaling leading to a shift in function from cytolytic to immunosuppressive[148]. Once viral replication bypasses cytolytic activity early during a reactivation, stimulation of CD8+ T cell expansion by CD4+ T cells and DCs is necessary for viral clearance. This delay may allow for enhanced viral spread before clearance[107]. Wide spatial dispersion of virus occurs during reactivation, which may serve to bypass areas of high T cell density[149].

Overall, these observations suggest a model of pathogenesis with the following features: first, due to focal HSV reactivation within sections of ganglia perhaps resulting from incomplete host immunological control, the virus leaks from neurons into the genital tract frequently, leading to recurrent, detectable shedding episodes (**Fig. 3**); second, the major determinant of episode severity is whether escape from innate and acquired immune surveillance occurs early during viral spread, as local immunologic control in the ganglia (**Fig. 4**) as well as genital keratinocytes may be altered by environmental stimuli; third, rapid containment of viral replication is required to prevent genital lesions; fourth, there seems to be a threshold of immune cell density above which control is achieved; and finally, these rapid interactions between virus and host are highly localized in time and space, and mucosal CD8+ and CD4+ T cell numbers slowly decline over time at a site of a prior recurrence (**Fig. 3**), allowing for subsequent herpetic lesion formation at the same site.

Implications for vaccines and immunotherapy

These new insights on viral-host interactions offer both opportunities and challenges for the development of new vaccine and immunotherapeutic approaches for HSV-2 infection. A successful vaccine product would limit the frequent leak of HSV-2 from dorsal root ganglia, as well as the extremely rapid expansion of virus early during episodes, both of which seem to occur despite multiple host control mechanisms within neural and mucosal tissue. Enhanced efficacy of antivirals may be obtained by precisely defining the pharmacodynamics and pharmacokinetics of the drugs in tissue. Effective delivery of drugs to the neural-epithelial cell junction may reduce initial seeding or localized bursts of initial rapid viral replication. The extremely quick expansion of virus early during reactivation, even in the presence of antiviral therapy, may imply that intracellular drug levels need to exceed the inhibitory concentration of drug throughout the dosing cycle.

An HSV-2 vaccine would have substantial beneficial effects at both the individual and population levels[150], and an ideal vaccine would significantly decrease the probability of acquisition per coital act. However, an immunotherapeutic vaccine that decreased disease severity and shedding frequency (and therefore transmission likelihood) would also be a welcome addition to the field. The recent demonstration that the host often controls HSV at the point of release, as well as through the frequent, rapid clearance of virus by the host immune system suggests potential for effective immunotherapy[50,136]. Given the importance of dense aggregates of lymphocytes and DCs in containing HSV within mucosa and possibly ganglia, an immunogen that increases mucosal innate immunity and HSV-specific T cell density and function appears to offer a reasonable chance of success in enhancing the localized containment of viral infection in tissue. The importance of these localized host responses in the clearance of HSV-2 infection suggests that measurement of mucosal HSV-specific CD8+ and CD4+ T cell density and function at the site of reactivation is likely to be a more fruitful correlate of clinical outcome than assessment of host immunity in peripheral blood mononuclear cells,

which may not reflect the host immune functions at localized tissue sites. Host immune cell density and functionality within dorsal root ganglia may also be crucial, though this will be considerably more difficult to assess from clinical samples.

In summary, recent studies indicate a nearly constant kinetic interaction between HSV-2 and the ganglionic and mucosal immune system, as HSV virions appear to leak frequently into the mucosa. The immunocompetent host is able to contain HSV-2 in a localized manner for extended time periods (weeks). The rapid expansion kinetics of reactivated virus and the wide anatomic distribution of virion release provide a favorable milieu for frequent shedding. The ability of the host to contain reactivation in a defined anatomic area for extended time periods provides promise for the development of immunotherapeutic approaches for viral containment in infected individuals as well as the development of immunogens to prevent transmission to sexual partners and from mothers to infants.

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The authors declare competing financial interests: details are available in the online version of the paper.

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