Review

Immune Control of HSV-1 Latency

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ABSTRACT

A hallmark of the herpes family of viruses is their ability to cause recurrent disease. Upon primary infection, Herpes Simplex virus (HSV) establishes a latent infection in sensory neurons that persists for the life of the individual. Reactivation of these latent viral genomes with virion formation is the source of virus for most HSV recurrent disease. This review details recent exciting findings supporting a role for the host immune system, particularly CD8+ T cells in maintaining HSV-1 in a latent state.

INTRODUCTION

Herpes Simplex Virus 1 (HSV-1) is a significant human pathogen, causing diseases ranging in severity from annoying labialis (lip lesions) and stomatitis (gum lesions) to blinding keratitis (corneal disease) and, rarely, lethal encephalitis (infection of the brain). Primary infection of mucosal surfaces following exposure to infected secretions results in a lytic infection in which more than 80 viral genes are sequentially expressed in a temporal cascade. Immediate early (α) gene products are primarily involved in transactivation of early and late genes and immune evasion; early (β) gene products are primarily involved in regulating viral DNA synthesis; and late (γ) gene products primarily represent viral structural proteins. The latter are subdivided into γ1 genes that are expressed early in the lytic cycle, but whose expression is enhanced after viral DNA synthesis; and γ2 genes that are only expressed after the initiation of DNA synthesis (31,53).

Primary infection with HSV-1 usually occurs early in life and is often subclinical. Most HSV morbidity results from recurrent disease, which is usually not initiated by exogenous reinfection. Instead, during primary infection the virus establishes a latent infection in sensory neurons, which then serves as a source of virus for recurrent disease. Although the mechanisms of HSV lytic infection are reasonably well understood, those resulting in the establishment, maintenance, and reactivation from latency are largely undefined. This review will address features of latency with a focus on the contribution of the host immune system to maintaining the viral genome in a latent state.

ESTABLISHMENT OF A LATENT INFECTION

During a primary infection, HSV-1 invades the neurons that innervate the infected mucosal surface. The virus is transported by retrograde axonal transport to the neuronal nuclei that are housed in the sensory ganglia. In animal models, HSV-1 replicates briefly in neurons, and...
progressive infection of surrounding neurons is observed. It has yet to be clarified if this process represents lateral neuron to neuron spread of the virus within the ganglion, or sequential invasion of neuronal termini with transport to cell bodies that are co-localized within the ganglion, or both (60). However, the fact that viral shedding can occur away from the site of primary infection suggests that some lateral spread must occur. After a brief period of replication the virus establishes a latent infection in which functional viral genomes are retained in neuronal nuclei without virus production.

Infection with virus that replicates normally in the periphery but poorly in the ganglia results in retention of very few copies of latent viral genome (32,67), although the number of copies per neuron may be unaffected (67). These observations are consistent with the notion that the level of virus replication in the ganglia during primary infection could be an important factor in determining the viral genome copy number in latently infected ganglia. Moreover, the number of copies of latent viral genome retained in ganglia is positively correlated with the reactivation frequency (56).

Thus, host factors that regulate HSV-1 replication in neurons during primary infection probably represent one factor that determines susceptibility to recurrent disease.

Although unique intrinsic properties of neurons may contribute to the control of HSV-1 replication, the host innate and adaptive immune systems undoubtedly provide a second level of protection. The initial control of HSV-1 replication within the trigeminal ganglion (TG) following corneal infection in mice clearly involves components of innate immunity. HSV-1 begins to replicate in the TG within 2–3 days of corneal infection (60). Concurrently, the TG is infiltrated by macrophages and γδ TCR+ T cells (39,61). The macrophages produce nitric oxide (NO) and tumor necrosis factor (TNF)-α, both with known antiviral activity (34). Depleting macrophages, blocking NO production, or neutralizing TNF-α during the first 5 days after HSV-1 corneal infection significantly increases viral titers and the number of infected neurons in the TG (34). These findings suggest that macrophages function within the TG to limit virus replication and lateral spread.

Depletion of γδ TCR+ T cells from mice during the first 7 days after corneal infection also increased virus replication and spread and eliminated most of the early interferon gamma (IFN-γ) production within the TG (34,59). IFN-γ not only possesses antiviral activity, but is also a potent activator of macrophages, inducing their production of NO and TNF-α (29). Thus, early control of HSV-1 replication and spread in the TG appears to involve an orchestrated response in which γδ TCR+ T cells activate macrophages to produce antiviral compounds.

The type 1 IFNs also appear important in the early control of HSV-1 replication in the TG. Type 1 IFNs are produced in the TG after HSV-1 corneal infection concurrent with HSV-1 transport to the ganglion (9). In addition, over-expression of IFN-α1 in astrocytes inhibited HSV-1 replication and establishment of a latent infection in the TG. Another study showed that in primary TG cultures transduced with an adenoviral vector expressing IFNβ HSV-1 replication was repressed in a dose-dependent manner (2). Taken together, these findings suggest that innate immunity is primarily responsible for controlling early HSV-1 replication in the TG following primary infection, which is not surprising in light of the fact that the bulk of viral replication occurs during the first 5 days after infection, a time when the adaptive immune response is developing in the lymphoid organs.

MAINTENANCE OF HSV-1 LATENCY

In humans and in some animal models (rabbit and guinea pig), HSV-1 sporadically reactivates from latency in sensory neurons, is transported by anterograde axonal transport, and is shed at peripheral sites, potentially leading to recurrent disease. HSV-1 establishes a latent infection in the sensory ganglia of mice, but does not spontaneously reactivate. However, exposure of humans, rabbits, and mice to emotional or physical stress or immunosuppression can induce HSV-1 reactivation and shedding at the periphery (47,48,54).

At this juncture it is important to clarify how latency will be defined within the context of the ensuing discussion. HSV latency is classically defined as the retention of a functional viral genome in the extended absence of virus particles. By this definition, latency terminates and reactivation occurs when any viral lytic genes are expressed. This definition makes sense from a clinical standpoint since recurrent disease can only occur following production of infectious virus. However, latency has also been defined in molecular terms as a state in which viral gene expression is limited to a family of transcripts known as latency associated transcripts (LATs). By this definition, reactivation occurs when any viral lytic gene is expressed. We favor the classical definition of latency because it accommodates transient or extended expression of a limited array of viral lytic genes within a latently infected neuron. This definition applies to all other members of the herpesvirus family, and we believe it is an appropriate definition for HSV latency as well.

IS HSV-1 LYTIC GENE EXPRESSION ASSOCIATED WITH STABLE LATENCY?

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latency. Latency associated transcripts (LATs) are the only abundant viral transcripts present in latently infected neurons (64). However, no LAT translation product has been reproducibly observed. HSV transcripts other than LATs are not usually detected in latently infected neurons by in situ hybridization (15,64). These findings lead to the general concept that the latent viral genome is both transcriptionally and translationally silent, allowing the latent virus to effectively hide from the host immune system. However, recent studies employing more sensitive detection methods show expression of HSV immediate early (α), early (β), and even late (γ) viral genes in latently infected mouse neurons (13,14,21,35). Moreover, viral antigens have been detected in latently infected trigeminal ganglia (TG) in vivo (21,55) and in latently infected neurons in ex vivo TG cultures (38). Thus, the concept that the virus is able to “hide” from the immune system during latency now appears less tenable.

IS THERE A ROLE FOR THE IMMUNE SYSTEM IN MAINTAINING HSV-1 IN A LATENT STATE?

There is a well-defined association between immunosuppression and HSV reactivation from latency. For instance, immunosuppression for treatment of cancer and transplantation or in HIV patients frequently results in HSV reactivation from latency and severe disseminated infections (48,54). Moreover, various forms of stress (more subtle and common stimuli for HSV reactivation) are also associated with varying degrees of systemic immunosuppression (47). The association of HSV-1 reactivation with immunosuppression, combined with the recently observed long-term retention of T lymphocytes and T cell-derived cytokines in latently infected sensory ganglia are suggestive of a role for T cells in maintaining HSV-1 in a latent state (25,33,66).

Although both CD4+ and CD8+ T cells are retained in the trigeminal ganglion during HSV-1 latency, the involvement of CD4+ T cells in regulating HSV-1 latency has yet to be explored. In contrast, CD8+ T cells have been implicated in control of latency in several viral models including HSV-1. CD8+ T cell function is regulated by a cognate interaction with an epitope comprised of an antigenic peptide bound to an appropriate MHC class I molecule on the surface of a target cell. For CD8+ T cells to directly regulate HSV-1 latency, the target cells would need to express MHC class I molecules, that CD8+ T cells do, indeed, detect viral epitopes on latently infected neurons, and that the interaction of CD8+ T cells with latently infected neurons prevents full reactivation and virion formation.

DO INFECTED NEURONS EXPRESS MHC CLASS I?

Neurons are among a very small number of cell types that do not normally express detectable MHC class I molecules. Yet, the concept that CD8+ T cells can directly monitor HSV-1 gene expression in latently infected neurons assumes expression of an MHC class I/viral peptide complex on the surface of the neuron. How then can one make these two concepts compatible? A clue comes from reports by Pereira et al. (49,50) demonstrating that sensory neurons express readily detectable MHC class I during acute HSV-1 infection. These investigators documented MHC class I expression on sensory neurons during and briefly following the period of HSV-1 replication within the sensory ganglia. This was consistent with the observation that termination of HSV-1 replication in the peripheral nervous system was dependent on CD8+ T cells (63). Interestingly, HSV-1 replication and the associated MHC class I expression did not appear to result in loss of viability of the neurons. In contrast, once latency was established in the sensory ganglia, MHC class I expression was no longer detectable on the latently infected neurons.

These findings invite speculation that HSV-1 lytic genes and MHC class I genes might be concordantly regulated in neurons. Evidence presented above and that to follow suggests that a low level of viral gene expression is permitted within latently infected neurons, though the level of protein expression in most neurons might be too low to detect by conventional means. A similar argument might be made for MHC class I expression. Since CD8+ T cell stimulation requires extremely low levels of epitope expression (as little as one MHC class I/peptide complex per cell), it is conceivable that viral epitopes are present on the surface of latently infected neurons at a very early stage in the reactivation process (52).

EVIDENCE FOR CD8+ T CELL CONTROL OF HSV-1 LATENCY

Although the immune response at the primary site of HSV-1 infection has been well characterized, less is known about the immunological events that occur in sensory ganglia during latency. However, substantial evidence is now emerging in support of a role for T lympho-
phocytes in controlling HSV-1 latency. Expression of the T cell chemoattractant RANTES as well as T cell-derived anti-viral cytokines (i.e., IFNγ and TNF-γ) is detectable in latently infected TG for at least 180 days after HSV-1 corneal infection (8, 12, 24, 25, 39). Moreover, histological analysis of mouse TG after HSV-1 corneal infection revealed that both CD4+ and CD8+ T cells accumulate and are retained in the ganglion seemingly for the life of the animal (39, 61). The CD8+ T cells were found in close apposition to the neuron cell bodies within the ophthalmic branch of the TG. The relevance of these findings in HSV-1-infected humans was revealed in a recent report showing localization of CD8+ T cells to neuron cell bodies in TG of humans with a history of recurrent HSV-1 infections (66). Importantly, the CD8+ T cells selectively associated with neurons that harbored latent HSV-1 to the exclusion of those harboring latent Varicella-Zoster virus.

The majority of the CD8+ T cells in latently infected TG of C57Bl/6 mice are specific for a single immunodominant epitope on the HSV-1 γ1 gene product glycoprotein H (gB498–505) (33). Interestingly, many of these gB498–505-specific CD8+ T cells acquired expression of the recent activation marker (CD69) while present in the latently infected ganglia, and polarized their T cell receptor (TCR) to the junction with neuron cell bodies. The latter findings provide strong evidence for the concept that low levels of certain viral proteins (in particular gB) are produced in neurons, and recognized by CD8+ T cells within the ganglion. It is reasonable to conclude, therefore, that CD8+ T cells can respond to viral gene expression in latently infected neurons prior to full reactivation and virus formation.

A role for CD8+ T cells in blocking HSV-1 reactivation from latency has received further support from studies incorporating ex vivo cultures of latently infected TG. In one such study, the CD8+ T cells that were present in latently infected TG of mice 14 days after HSV-1 corneal infection were shown to completely inhibit reactivation in ex vivo TG cultures (38). In addition, a gB498–505-specific CD8+ T cell clone was shown to block HSV-1 reactivation from latency in a dose-dependent, antigen-specific, and MHC-restricted fashion when added to CD8+ T cell-depleted ganglion cultures (33). The protected cultures were shown by RT-PCR to lack transcripts for the viral y2 gene for glycoprotein H (gH). These studies demonstrated an exquisite CD8+ T cell regulation of HSV-1 gene expression during latency in which recognition of a γ1 gene product, gB, induced a response that terminated the viral life cycle prior to expression of the γ1 gene, gH.

A role for T cells in controlling viral latency has been observed in other virus models such as cytomegalovirus (CMV) infection and gammaherpesvirus 68 (γHV68). The adoptive transfer of HCMV-specific CD8+ T cells to human allogeneic bone marrow recipients was shown to inhibit HCMV reactivation from latency (69). These findings were expanded in a mouse model. Studies in B cell-deficient mice with latent CMV infections established a hierarchy of control by CD8+ T cells, NK cells, and CD4+ T cells. Reactivation of latent CMV was rarely observed following individual depletion of CD8+ T cells or CD4+ T cells, but was evident after depletion of both T cell subpopulations. Moreover, depletion of either CD4+ or CD8+ T cells alone in conjunction with neutralization of IFN-γ efficiently induced reactivation, suggesting that both T cell subpopulations used IFN-γ to inhibit CMV reactivation (51). Similarly, CD8+ T cells can inhibit γHV68 reactivation from latency (68). However, in this model lytic granule exocytosis appears to be an essential effector mechanism used by CD8+ T cells to block reactivation (40).

As noted previously, IFN-γ mRNA and proteins are consistently detected in ganglia latently infected with HSV-1 (8, 25, 39, 62). Although HSV-1 does not spontaneously reactivate in IFN-γ−/− or IFN-γ−/− mice, the incidence of stress-induced reactivation in these mice was significantly higher than that of wild type mice (7, 44). While the results of this study suggested a role for IFN-γ in preventing HSV-1 reactivation from latency in vivo, the investigators did not rule out the alternative possibility that the increased susceptibility to reactivation resulted from a higher copy number of viral genomes in the sensory neurons of IFN-γ−/− and IFN-γR−/− mice. However, subsequent in vitro studies demonstrated that IFN-γ could block HSV-1 reactivation from latency when added to cultures of latently infected TG early in the reactivation process (37). Recent findings from our laboratory suggest that IFN-γ can block multiple steps in the viral life cycle during reactivation from latency in some neurons, whereas other neurons appear to be refractory to the inhibitory effect of IFN-γ (unpublished data).

Emerging data support the theory that the net effect of cross-regulation between IFN-γ and the HSV-1 gene product, IC0, might influence the balance between HSV-1 latent and lytic infection. IC0 is a promiscuous transactivator required for efficient HSV-1 reactivation from latency (26, 27). Early after infection, HSV-1 genomes associate with cellular nuclear domain 10 (ND10) bodies (6, 42). Viral gene transcription and DNA replication occur in globular replication compartments whose formation requires prior dissociation of ND10 bodies (18, 19). IC0 targets the PML component of ND10 bodies for destruction in proteasomes, augmenting HSV-1 gene expression and virus formation. It has been proposed that IC0 regulated destruction of ND10 bodies might also facilitate HSV-1 reactivation from latency (20). In addi-
tion, ICP0 facilitates HSV-1 replication by targeting IFN-induced antiviral proteins for destruction by proteasomes (10,36).

IFN-γ counters the effects of ICP0 in several ways. During lytic infection, IFN-γ inhibits production of ICP0 transcripts (28,65) and strongly up-regulates expression of the various components of ND10 bodies (11,23). IFN-γ also induces the production of the cyclin-dependent kinase (cdk) inhibitors p21WAF1(CIP1) and p27KIP1, resulting in decreased cdk2 and cdk4 activity (41). Work of Schaffer and colleagues demonstrated that activities of these kinases are required for HSV-1 replication, and α and β gene expression (57,58). Furthermore, cdk2 is required for ICP0 postranslational modifications necessary for its transactivating activity (17), but does not affect the ability of ICP0 to degrade the components of ND10 bodies (10). Together, these findings suggest a model in which the presence of IFN-γ early in the reactivation process might block the functions of ICP0 that are required for HSV-1 reactivation from latency. A delay in the presence of IFN-γ would allow ICP0 protein to accumulate in the latently infected neuron, enhancing expression of viral lytic cycle genes, and inhibiting IFN-γ function.

As noted above, the addition of IFN-γ to ex vivo cultures of latently infected TG can block HSV-1 reactivation in some, but not all neurons. In contrast, the addition of HSV-1-specific CD8+ T cells to such cultures blocks reactivation in all neurons. These findings demonstrate that CD8+ T cells employ more than one effector mechanism in blocking HSV-1 reactivation from latency, and that individual latently infected neurons differentially respond to these effector molecules. The identity of the other CD8+ T cell effector molecules that inhibits HSV-1 reactivation from latency remains to be determined.

WHY DO SOME PEOPLE GET RECURRENT HERPETIC DISEASE IN THE FACE OF A ROBUST HSV-1 SPECIFIC CD8+ T CELL RESPONSE?

As noted above, CD8+ T cells in both mice and humans are retained in the TG in close apposition to latently infected neurons, and murine CD8+ T cells can completely block HSV-1 reactivation from latency in ex vivo TG cultures. Why then is recurrent herpetic disease so prevalent? At least two explanations present themselves. One possible explanation lies in the fact that the HSV-1a gene product ICP47 can block the transport of peptides into the endoplasmic reticulum for loading on MHC class I (22,30). The effect of ICP47 on HSV-1 epitope recognition on neurons might be especially profound given that these cells normally express very low to undetectable levels of MHC class I. In that regard, it is interesting to note that (a) ICP47 blocks TAP transport of peptides far more effectively in human cells than in mouse cells, and (b) HSV-1 spontaneously reactivates from latency in humans but not or at a very low frequency in mice. It is conceivable that the propensity of HSV-1 to reactivate in humans is related to an inhibitory effect of ICP47 on epitope expression on human neurons.

Stress might be another factor that influences the susceptibility of some people to recurrent herpetic disease. Glucocorticoids are effector molecules of the hypothalamic–pituitary axis (HPA). Corticosterone is the major HPA axis-derived hormone in mice and it influences T cell function in a variety of ways. Corticosterone regulates production of a variety of cytokines that influence T cell migration and proliferation (4,43,46); regulates IL-2 receptor α and β and IL-6 receptor expression (3); induces T cell apoptosis; and blocks the T cell cycle at G0/G1 (45).

Changes in T cell survival or localization due to glucocorticoids may be sufficient to allow HSV to reactivate from latency. Restraint stress during latency has been shown to decrease lymphocyte numbers in the spleen and lymph nodes as well as disrupt the ability of CD8+ T cells to lyse HSV-infected target cells (5). Restraint stress during latency has also been associated with a reduction of IL-2, IL-4, IL-6, and IFNγ production by splenic lymphocytes in response to HSV-1 antigens (4). In addition, glucocorticoids might directly influence viral gene expression in latently infected neurons. Activated glucocorticoid receptor binding to CREB binding protein (CBP) can enhance an endogenous histone acetylation activity of CBP (1). This could allow for increased transcription of DNA sequences of the latent viral genome to become accessible to transcription factors. Thus, stress might lead to HSV-1 reactivation from latency in humans by transiently compromising the function of CD8+ T cells within the sensory ganglia, and by enhancing viral gene expression within latently infected neurons.

Based on the above discussion, we conclude that a bivalent interaction between a neuron and the HSV-1 genome probably does not constitute a full representation of HSV-1 latency. Instead, some neurons appear to require a tripartite interaction among the neuron, the viral genome, and the host immune system. Moreover, we further propose that maintaining the virus in a latent state in certain neurons is accomplished through the activity of IFN-γ and at least one other CD8+ T cell effector mechanism. Defining the role of T lymphocytes in maintaining HSV-1 latency and identifying the viral antigens that stimulate their activity might lead to new vaccine-based approaches to preventing recurrent herpetic disease.
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