Published in final edited form as:

J Mol Biol. 2014 March 20; 426(6): 1133–1147. doi:10.1016/j.jmb.2013.11.012.

Recognition of Herpes Simplex Viruses: Toll-like Receptors and Beyond

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Abstract

Herpes simplex viruses (HSV) are human pathogens that establish lytic and latent infections. Reactivation from latency occurs intermittently, which represents a life-long source of recurrent infection. In this complex process, HSV triggers and neutralizes innate immunity. Therefore, a dynamic equilibrium between HSV and the innate immune system determines the outcome of viral infection. Detection of HSV involves pathogen recognition receptors which include Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I) like receptors, and cytosolic DNA sensors. Moreover, innate components or pathways exist to sense membrane fusion upon viral entry into host cells. Consequently, this surveillance network activates downstream transcription factors, leading to the induction of type I interferon (IFN) and inflammatory cytokines. Not surprisingly, with the capacity to establish chronic infection HSV has evolved strategies that modulate or evade innate immunity. In this review, we describe recent advances pertinent to the interplay of HSV and the induction of innate immunity mediated by pathogen recognition receptors or pathways.

Keywords

Herpes simplex virus; Toll-like receptor; interferon; cytokine; innate immunity

Introduction

Herpes simplex virus (HSV) is a large DNA virus which belongs to the family of *herpesviridae*. Two serotypes HSV-1 and HSV-2 are responsible for various clinical manifestations, including cold sores, fever blisters, genital ulcers, blindness or encephalitis^{1,2}. HSV typically infects the mucosal tissues where the virus undergoes gene expression, DNA replication, assembly and egress. This involves sequential expression of viral proteins that act coordinately, leading to a productive cycle. Remarkably, HSV penetrates to the nervous system and establishes latency in sensory neurons¹. Viral reactivation occurs periodically, which is responsible for recurrent infections. In these complex processes, HSV triggers innate immunity, particularly type I IFN responses².

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The innate recognition of HSV initiates via Toll-like receptors (TLRs) and cytosolic receptors that detect invariant molecular structures termed pathogen-associated molecular patterns (PAMPs)^{3,4}. These receptors, namely pattern recognition receptors (PRRs), survey the cell surface and intracellular compartments for virally-derived DNAs, RNAs and proteins (Figure 1). Once engaged with viral ligands, TLRs and cytosolic receptors transmit signals to downstream adaptor proteins such as myeloid differentiation primary response protein 88 (MyD88), Toll/interleukin-1 receptor (TIR) domain-containing adaptor inducing IFN- β (TRIF), mitochondrial antiviral-signaling protein (MAVS) and stimulator of IFN genes (STING; also known as Mediator of IRF3 activation (MITA)⁵ or Endoplasmic Reticulum IFN Stimulator (ERIS)⁶). This leads to the formation of distinct protein complexes that activate the classical inhibitor of kappa B (I- κ B) kinase (IKK) and closely related kinases known as TANK-binding kinase 1 (TBK1) and inducible IKK kinase (IKKi). Accordingly, I- κ B kinase (the IKK $\alpha\beta\gamma$ complex) activates the transcription factor NF- κ B, which translocates to the nucleus and induces the expression of inflammatory cytokines. In parallel, TBK1/IKKi activates transcription factors interferon regulatory factor 3 (IRF3) and interferon regulatory factor 7 (IRF7), which induce the expression of IFN- α/β and chemokines. Additionally, HSV stimulates the assembly of inflammasomes and activates caspase-1, which in turn proteolytically cleaves pro-interleukin 1 β (pro-IL-1 β) and pro-IL-18 for maturation⁷. Collectively, type I IFNs and cytokines exert anti-viral and immunomodulatory activities in an autocrine, paracrine or endocrine manner.

A complex cellular program operates in response to HSV infection, which is cell-type specific and time-dependent^{8,9}. Although TLR pathways recognize HSV, additional pathways exist to mediate type I IFN responses^{3,10,11}. Furthermore, inflammasome pathways detect HSV as well⁷. These innate immune pathways form a barrier to invading HSV. Meanwhile, as a human pathogen HSV has evolved strategies of immune evasion¹². In this review, we discuss recent progress on HSV induction and evasion of innate immunity involving type I IFN and inflammasome pathways.

TLR-Mediated HSV Recognition

TLRs are type 1 transmembrane proteins that recognize PAMPs. They signal via MyD88-dependent or TRIF-dependent pathways. TLR 1, 2, 4, 5, and 6 are expressed on the cell surface whereas TLR 3, 7, 8 and 9 are located on the endosomal membranes and detect virally-derived nucleic acids^{13,14}. The patterns of TLR expression vary among cell types with haematopoietically derived cells such as macrophages and dendritic cells (DCs) expressing nearly all TLRs. With respect to HSV infection, it is notable that TLRs are expressed differentially in the epithelial cells from HSV-targeted oral, ocular and genital mucosa¹⁵ as well as in the central nervous system (CNS) resident cells^{16,17}.

TLR2

TLR2 recognizes microbial lipopeptides and induces inflammatory cytokines in a MyD88-dependent manner¹³. During HSV infection, viral glycoproteins on the envelope serve as PAMPs for TLR2. A recent study has shown that HSV glycoproteins gH/gL and gB are able to interact with TLR2¹⁸. While each acts as a ligand to TLR2, gH/gL, but not gB, is sufficient to activate NF- κ B¹⁸. Moreover, gH/gL-mediated TLR2 signaling involves α V β 3-integrin¹⁹. In a separate study gB is reported to bind TLR2 and activate NF- κ B through the MyD88/TRAF6-dependent signaling pathway²⁰. This discrepancy with respect to gB might stem from differences in experimental systems. Thus, engagement of TLR2 with multiple viral glycoproteins may favor ligand recognition or signal transduction. As gH/gL and gB are virion components, they contribute to cytokine expression during viral entry, an early stage of HSV infection. The precise nature of interactions between TLR2 and viral glycoprotein awaits further investigation.

TLR2 activation has a differential role in HSV pathogenesis. A high mortality in neonatal mice infected with HSV-1 is linked to the detrimental effect of inflammatory responses mediated by TLR2²¹. TLR2 promotes the production of inflammatory cytokines and chemokines in primary microglial cells during HSV-1 infection, suggesting that TLR2 plays a role in immunopathology of HSV infection²². Similarly, TLR2 mediates early inflammatory events in stromal keratitis²³. Polymorphisms in TLR2 also correlates with increased disease severity in patients with genital HSV-2 infection²⁴. Consistently, clinical isolates of HSV-1 and HSV-2 induce inflammatory cytokines and type I IFNs in DCs via TLR2 and TLR9²⁵. On the other hand, TLR2 is required to reduce the viral load in trigeminal ganglia (TG) or brain in HSV infection and such viral control requires TLR9 for maximal synergy^{26,27}.

TLR3

TLR3 is well established for its ability to recognize dsRNA and induce the expression of type I IFNs and inflammatory cytokines³. It is localized to the endosome and relies on the adaptor TRIF and TRAF3 for downstream signaling. It is not clear how HSV activates TLR3. However, it has been shown during HSV infection viral gene expression proceeds in a temporal fashion where viral dsRNAs are generated²⁸. Although details are unknown, viral dsRNAs are detectable in HSV-infected cells²⁹. Therefore, these viral dsRNAs presumably serve as the ligand for TLR3.

TLR3 mediates the type I IFN response that is cell type dependent upon HSV infection^{30,31}. Human peripheral blood mononuclear cells (PBMCs) and fibroblasts isolated from TLR3-deficient patients respond to HSV-1 differently^{30,31}. When stimulated with dsRNA or HSV, TLR3-deficient PBMCs effectively produces type I/III IFN whereas TLR3-deficient fibroblasts are unable to do so. Several TLR3-deficient leukocyte subsets also behave normally like PBMCs³⁰. Notably, HSV replicates more efficiently in TLR3 deficient neurons where type I IFN production is impaired³². In murine astrocytes as well as DCs, TLR3 also acts to limit HSV infection^{33,34}. Compared to wild type cells, astrocytes lacking TLR3 produce a higher level of HSV. This parallels with the defective type I IFN production. However, TLR3 signaling is dispensable for type I IFN production in response to HSV infection in murine macrophages³⁵. Therefore, TLR3 is largely redundant in some cell types where other pathways may compensate for TLR3 deficiency in HSV-infected cells.

It is noteworthy that TLR3 has a protective role against HSV, particularly in the CNS^{30,31,33,34}. In human, TLR3 deficiency predisposes children to herpes simplex virus encephalitis (HSE)^{30,31}. A heterozygous mutation in the ligand binding domain of TLR3 is linked to HSE. This single amino acid substitution (P554S), located within a region required for dsRNA binding and dimerization, results in a dominant negative phenotype. In addition, two compound heterozygous mutations account for a complete loss of TLR3 function. In this case, one allele bears P554S substitution whereas the other has a nonsense mutation (E746X) that prevents translation of the TIR domain of TLR3. Evidence supports a model that the TLR3 axis consisting of UBC93B, TRIF, TRAF3 and TBK1 exerts protective immunity to HSV-1 in the CNS^{30,36,37,38}. Nevertheless, these TLR3 deficient individuals who are succumbed to HSE are free of clinical HSV-1 dissemination and other viral infections, suggesting that TLR3 is dispensable for antiviral immunity in the peripheral system. One study with a murine model suggests that TLR3 precludes HSV-2 entry into the CNS through astrocyte-mediated IFN response³⁴. Therefore, TLR3 displays a unique activity to control HSV infection in the CNS.

TLR9

TLR9 recognizes dsDNA containing un-methylated CpG motifs in endosomes³. Both HSV-1 and HSV-2 infections can stimulate IFN- α production in plasmacytoid dendritic cells (pDCs), which requires TLR9, MyD88 and an intact endocytic pathway but not viral replication^{39,40}. HSV genomic DNA contains abundant un-methylated CpG motifs that induce early type I IFN response mediated by TLR9 in pDCs^{8,39}. In addition, TLR9 contributes to the induction of type III IFN in response to HSV infection in DCs although this relies more on NF- κ B than IRFs which are critical for type I IFN transcription⁴¹. Nonetheless, the requirement of TLR9 for HSV-induced innate immunity appears cell type-specific⁸. TLR9 or MyD88 knockout mice are capable of controlling HSV-1 replication *in vivo*, indicating that other HSV recognition pathways compensate for the loss of TLR9/MyD88 signaling⁴⁰. Consistent with this, HSV induces TLR9-independent production of cytokines and type I IFNs in pDCs, conventional DCs (cDC), macrophages and fibroblasts^{8,9,26,35,40}. These studies suggest that TLR9 only partially contributes to the innate antiviral responses to HSV in cells other than pDCs.

Recognition of HSV by Cytosolic RNA Receptors

RIG-I-like receptors (RLRs)

Belonging to the DExD/H-box RNA helicase family, RIG-I and melanoma differentiation-associated gene 5 (MDA5) are known as RLRs⁴². RLRs elicit antiviral immunity upon detecting RNA in the cytosol. RIG-I recognizes RNA bearing 5'-triphosphate group whereas MDA5 recognizes long dsRNA with higher ordered structures^{43,44,45,46}. RLRs transmit signals to the adaptor MAVS (also known as virus-induced signaling adaptor (VISA), interferon β promoter stimulator (IPS-1), and caspase recruitment domains (CARD) adaptor inducing IFN- β (Cardif)) which activates IRF3 and NF- κ B, resulting in expression of type I IFNs and inflammatory cytokines⁴². In HSV-infected cells, viral replication generates RNAs^{28,29}, which are thought to trigger RLRs.

The connection between HSV and RIG-I is initially suggested by the observation that HSV-1 replicates robustly in human hepatoma cells line lacking a functional RIG-I⁴⁷. In murine macrophage cell lines, the dominant negative mutant of RIG-I drastically reduces type I IFN production in response to HSV-2 infection⁴⁸. Furthermore, IFN- α/β production induced by HSV infection is impaired in embryonic fibroblasts derived from MAVS knockout mice^{8,48}. However, knockout of MAVS does not affect inflammatory cytokine expression in cDCs in HSV-1 infection, indicating that RLR/MAVS signaling is not essential for the DC response to HSV-1⁴⁹. Recently, a role of MDA5 in HSV recognition is also reported⁵⁰. Data suggest that MDA5, but not RIG-I, is the primary sensor responsible for HSV-1-triggered type I/III IFN and cytokine production in human primary macrophages⁵⁰. The HSV RNA ligand(s) for RLRs in infected cells have not been identified. Deletion analysis reveals that RNA transcripts from HSV immediate early genes, including $\alpha 4$, $\alpha 22$, and $\alpha 27$ are not involved⁸. The precise mechanism by which RLRs detect HSV requires further investigation. Recent evidence suggests that additional cytosolic RNA receptors exist^{51,52}. A cytosolic RNA complex, consisting of helicases DEAD (Asp-Glu-Ala-Asp) box polypeptide 1 (DDX1), DDX21, and DEAH (Asp-Glu-Ala-His) box polypeptide 36 (DHX36), has been reported to bind poly(I:C) and recruit TRIF to activate the type I IFN response in DCs⁵¹. Furthermore, DHX9 cooperates with MAVS to detect cytosolic RNA from RNA viruses in DCs⁵². It is yet to be determined whether these receptors play a role in sensing HSV.

Cytosolic DNA Receptors and HSV Recognition

Recognition of intracellular DNA operates independently of TLRs, where B-DNA or Z-DNA activates IRF3 in a pathway involving TBK1^{53,54}. STING functions as a key adaptor downstream of majority of cytosolic DNA sensors that leads to the type I IFN response^{55,56}. A growing body of evidence suggests that intracellular receptors sense DNA in the cytosol and induce type I IFN and inflammatory cytokines (Figure 2). Among them, DNA-dependent activator of IFN-regulatory factor (DAI), cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (cGAS), DDX41, DNA dependent protein kinase (DNA-PK), and IFN- γ -inducible protein (IFI16) signal through the STING-TBK1 axis in response to HSV infection^{57,58,59,60,61}. Although relying on STING, meiotic recombination 11 homolog A (MRE11) is not required for HSV recognition⁶². RNA polymerase III (PolIII) transcribes DNA to RNA that acts as a RIG-I ligand^{63,64}. Leucine-rich repeat flightless-interacting protein 1 (LRRFIP1) mediates the induction of type I IFN via a β -catenin dependent pathway but its role in HSV recognition is to be established⁶⁵. In addition, DHX9, DHX36, and Ku70 relay signals to activate IRF7 and IRF1 upon HSV infection^{66,67}. Notably, IFI16 also forms inflammasome⁷.

DAI

DAI, also known as Z-DNA-binding protein 1 (ZBP-1) and DLM-1, is the first cytosolic DNA receptor reported to recognize HSV⁵⁷. DAI binds to either B-form or Z-form DNA from a variety of sources and activates TBK1 and IRF3, culminating in the type I IFN response^{57,68}. DAI is also observed to recruit receptor interacting protein 1 (RIP1) and RIP3 through its RIP homotypic interaction motifs to activate NF- κ B pathway⁶⁹. In L929 cells, a murine aneuploid fibrosarcoma cell line, DAI mediates the production of IFN- β in response to HSV-1 but not RNA viruses such as the Newcastle disease virus (NDV)⁵⁷. However, studies using RNAi knockdown or DAI-deficient mice show that DAI-mediated DNA recognition is dispensable for DNA-induced immunity^{57,68,70}, suggesting the existence of additional cytosolic DNA-sensing pathways. A study using primary murine microglia and astrocytes has suggested that DAI mediates inflammatory responses of resident CNS cells to HSV-1 challenge⁷¹. A separate study has shown that DAI is involved in suppression of viral genomes and regulation of ICP0 protein of HSV-1 independently of type I IFN response⁷². This reveals an additional role of DAI in controlling HSV-1 infection. Interestingly, DAI forms complex with RIP3 and sensitizes cells to virus-induced programmed necrosis during murine cytomegalovirus infection⁷³. Whether this requires the ability of DAI to sense DNA is unknown.

RNA PolIII

RNA PolIII detects cytosolic DNA, which relies on RIG-I^{63,64}. It transcribes AT-rich DNA into dsRNA containing 5'-triphosphate moiety, which triggers the type I IFN response. Studies suggest that RNA PolIII is responsible for type I IFN production in HSV-infected murine macrophage cell lines. Notably, an inhibitor of RNA PolIII reduces IFN- β induction by HSV-1⁶³. However, other studies have suggested that inhibition of RNA PolIII has no effect on the induction of type I IFN and TNF- α in macrophages infected with HSV-1^{50,59}. The basis for the observed difference between these studies is unclear.

IFI16

Human IFI16 protein and its murine homologue Ifi204 (p204) belong to the PYHIN protein family, with an amino-terminal pyrin (PYD) domain and one or two carboxy-terminal HIN200 domains that are capable of DNA binding⁷⁴. IFI16/p204 has been identified as an intracellular DNA sensor to induce type I IFN production⁵⁹. IFI16/p204 binds cytosolic dsDNA and induces type I IFNs and inflammatory cytokines through STING^{75,76}. RNAi

knockdown of IFI16/p204 in human and murine monocytic cell lines abrogates the activation of IRF3 and NF- κ B and subsequent induction of IFN- β and inflammatory cytokines⁵⁹. When pretreated with HSV 60mer DNA, murine macrophage cells mount antiviral immunity that potently suppresses HSV-1 replication. In line with this, IFI16/p204 is reported to mediate mucosal immunity against HSV-1 and HSV-2 *in vivo* and epithelial cell cultures⁷⁷. Therefore, IFI16/p204 is a restricting factor for HSV-1 replication.

IFI16 is detectable in the nucleus, cytoplasm, or both, depending on cell types⁷⁸. This protein contains a multipartite nuclear localization signal (NLS) that undergoes acetylation in lymphocytes as well as in macrophages⁷⁹. Intracellular acetyltransferases or deacetylases such as p300 regulate IFI16 acetylation and thus its cellular localization. Evidence suggests a model that recognition of HSV by IFI16/p204 may involve multiple mechanisms^{59,80,81}. In macrophages, IFI16 is mainly localized in the nucleus with a minor fraction in the cytoplasm. Therefore, IFI16 possibly senses viral DNA in both compartments during HSV infection⁸². It has been reported that recognition of HSV by IFI16 in human macrophages requires proteasomal degradation of viral capsids, which releases HSV DNA into the cytosol⁸⁰. In contrast, in human foreskin fibroblasts IFI16 resides exclusively in the nucleus and recognizes viral DNA accumulated during productive HSV-1 infection⁸¹. Consistently, IFI16 primarily detects HSV-1 DNA in the nucleus of U2OS cells⁷⁹. These studies raise a question as to how nuclear IFI16 delivers signals to the cytosolic adaptor STING, which is amendable for further exploration.

DExD/H-box RNA helicases

Several DExD/H-box RNA helicase family members have emerged as cytosolic DNA sensors, which include DHX9, DHX36, and DDX41^{60,66}. In a human pDC cell line, DHX36 and DHX9 sense cytosolic CpG-A and CpG-B DNA, respectively⁶⁶. When stimulated with ligands, both DHX36 and DHX9 interact with adaptor MyD88, which activates IRF7 and NF- κ B, leading to production of type I IFNs and inflammatory cytokines. In HSV-1 infected human pDCs, siRNA knockdown of DHX36 impairs IFN- α production whereas knockdown of DHX9 inhibits TNF- α production, suggesting DHX36 and DHX9 are involved in HSV-1 induced type I IFN induction and inflammatory cytokine expression, respectively⁶⁶.

In addition, it has been suggested that DDX41 is a cytosolic DNA receptor in both murine DCs and human monocytes⁶⁰. Upon recognition of transfected dsDNA or DNA virus infection, but not RNA virus infection, DDX41 interacts with STING and activates the STING-TBK1-IRF3 axis, leading to type I IFN induction. Moreover, DDX41 triggers MAPK signaling pathway⁶⁰. In murine DCs and human monocytic cells, the DDX41/STING-dependent pathway mediates antiviral immunity against HSV-1⁶⁰. Upon stimulation with HSV-1 DNA, DDX41 and STING migrate from mitochondria and mitochondria-associated endoplasmic reticulum membranes to microsomes. Knockdown of DDX41 or STING cripples the production of type I IFNs and inflammatory cytokines in response to HSV-1 infection. These results suggest that DDX41 serves as a cytosolic sensor to recognize HSV-1 infection.

Ku70/DNA-PK

The induction of type III IFN production by transfection of non-coding plasmid has led to the identification of Ku70 as a cytosolic DNA sensor⁶⁷. Knockdown of Ku70 inhibits the expression of IFN- λ 1 and RANTES in HEK293 in response to linearized plasmid DNA. This requires IRF1 and IRF7 rather than IRF3. A subsequent study has demonstrated that the heterotrimeric protein complex DNA-PK, which consists of Ku70, Ku80 and the catalytic subunit DNA-PKCs, is a cytosolic DNA receptor and induces the expression of type I IFNs

and other cytokines in fibroblasts, which requires STING and IRF3 in the downstream signaling⁶¹.

The role of Ku70 in sensing HSV is suggested by the observation that siRNA knockdown of Ku70 significantly impairs transcription of IFN- λ 1 in HSV-2 infected cells⁶⁷. Importantly, IL-6 cytokine expression is suppressed in mice lacking components of DNA-PK despite the presence of other DNA sensors, such as DAI, PolIII, IFI16 and DDX41. These studies suggest that Ku70/DNA-PK is a critical cytosolic sensor recognizing HSV infection, presumably by detecting HSV genomic DNA. Studies have shown that DNA viruses such as HSV induce host DNA-damage response, where DNA-PK is a critical mediator⁸³. Moreover, a recent study has linked the DNA-damage response to the activation of type I/III IFNs⁸⁴. In this regard, the finding that Ku70/DNA-PK mediates the antiviral response provides a logical explanation. Notably, another DNA damage sensor, MRE11, is also reported to recognize dsDNA and induce type I IFNs by regulating STING trafficking⁶². Nevertheless, knockdown of MRE11 does not impair type I IFN production in response to HSV-1 infection. These observations imply that components of DNA repair machinery may have an integrated function in DNA-damage and innate antiviral responses.

cGAS

cGAS catalyzes cGAMP synthesis in a DNA dependent manner⁵⁸. A recent study shows that delivery of DNA to mammalian cells or cytosolic extracts stimulates the production of cGAMP, which binds to and activates STING, leading to IFN- β induction⁸⁵. Notably, HSV-1 as well as vaccinia virus triggers cGAMP production and subsequent IRF3 activation in murine L929 and human monocyte cell lines⁸⁵. These data argue that cGAMP can act as a second messenger to mediate the type I IFN pathway in response to cellular or viral DNA. Further investigation through biochemical analysis has revealed that the cGAMP synthase, cGAS, is a cytosolic DNA sensor that activate type I IFN responses⁵⁸. cGAS, predominantly localized in the cytoplasm, binds to DNA via its amino-terminal domain. Its depletion markedly reduces the level of cGAMP and IRF3 activation in response to HSV-1 infection. Therefore, cGAS functions as a DNA sensor that recognizes HSV in infected cells.

Membrane Fusion and HSV Recognition

Although PRRs recognize PAMPs, an additional receptor(s) or pathway(s) senses the virus-cell membrane fusion event^{11,86}. It has been reported that HSV entry, in the absence of viral replication, induces a subset of interferon-stimulated genes (ISGs) in human fibroblasts^{87,88}. This early response is abolished in IRF3 $^{-/-}$ and TBK1 $^{-/-}$ mouse embryonic fibroblasts but remains normal in wild type, TRIF $^{-/-}$ or MyD88 $^{-/-}$ cells^{10,89}. These observations suggest that viral entry activates TBK1 that phosphorylates IRF3 independently of TLR pathways. However, NF- κ B is not activated, which is consistent with the lack of inflammatory cytokine production^{10,87}. Available data suggest that the phosphoinositide-3-kinase (PI3kinase) is required in this process to elicit antiviral immunity^{11,90}. This is supported by the observation that an inhibitor of PI3kinase precludes the induction of ISGs upon HSV entry. Hence, PI3kinase may cooperate with STING and TBK1 to initiate innate immune signaling.

HSV entry involves fusion of viral envelope with the cell membrane. HSV-1 particles, which lack glycoprotein D or glycoprotein H, are unable to elicit ISG induction⁸⁸. This implies that viral penetration rather than binding to the cell surface is responsible for ISG induction. Membrane perturbation has been shown to elicit IRF3-dependent antiviral response⁸⁶. One study has demonstrated that HSV-cell fusion induces STING-dependent type I IFN response¹¹. Virus-like particles (VLP) which are devoid of the HSV genome induce the expression of type I IFNs and chemokines. However, when glycoprotein B or

glycoprotein H is absent in these HSV genome-deficient VLPs, they are unable to induce the expression of type I IFNs and chemokines, suggesting that the recognition of VLPs by the immune system depends on fusion. It is notable that VLP-triggered response proceeds normally in TRIF^{-/-}, MyD88^{-/-}, MAVS^{-/-}, TLR2^{-/-}TLR9^{-/-}, and TLR3^{-/-} cells. Furthermore, liposome as well as HIV-1 Env-mediated fusion stimulates the type I IFN response. Thus, viral-cell fusion stimulates TLR and RLR-independent innate immunity. While the sensor is unknown, these studies support the concept that in addition to molecular signatures associated with viral pathogens, the innate immune system also senses a biological process associated viruses or host cells.

Inflammasome-Mediated HSV recognition

Inflammasomes are multi-protein complexes which control the activation of caspase-1 and maturation of IL-1 β and IL-18 in response to a range of stimuli⁹¹. Of all inflammasomes identified so far, NOD-like receptor (NLR) family, pyrin domain containing 3 (NLRP3) and IFI16 have been shown to recognize herpesviruses^{7,82,92,93}. It has been reported that IFI16 activates inflammasome upon human herpesvirus 8 (HHV-8) and Epstein-Barr virus (EBV) infection^{82,93}. Unlike absent in melanoma 2 (AIM2), a cytosolic DNA receptor that activates inflammasome in the cytoplasm⁹¹, IFI16 senses the HHV-8 genome and assembles a functional inflammasome in the nucleus, resulting in caspase-1 activation and processing of IL-1 β in the cytoplasm. Therefore, IFI16 not only mediates type I IFN induction but also inflammasome activation. However, in a separate study IFI16 is shown to mediate the anti-inflammatory effect of type I IFNs and suppress the activation of dsDNA-induced AIM2 and alum-induced NLRP3 inflammasomes⁹⁴. IFI16 binds to AIM2 in the cytoplasm and inhibits AIM2-mediated activation of caspase-1. Therefore, the role of IFI16 in innate antiviral responses is complex and likely to be context-dependent. Although HSV-1 infection induces IL-1 β maturation and release⁹⁵, evidence suggests that AIM2 does not play a role in this process⁹⁶. In contrast, IFI16 recognizes the HSV-1 genome in the nuclei of infected cells and translocates to the cytoplasm to form inflammasome with ASC⁷. The molecular basis for this distinct requirement in AIM2 and IFI16 inflammasome assembly is unclear.

NLRP3 is a NOD-like receptor that forms an inflammasome with ASC and caspase-1⁹¹. A wide range of signals of microbial and endogenous origins such as bacterial pore-forming toxins, uric acid, dsRNA and ATP, can activate the NLRP3 inflammasome. However, a specific ligand for NLRP3 has not been identified. Intriguingly, dsRNA-dependent protein kinase PKR is an upstream adaptor of NLRP3⁹⁷. Activation of inflammasomes requires two signals. The first signal activates PRRs (TLRs and NLRs) or cytokine receptors leading to up-regulated expression of pro-IL-1 β and other components of the inflammasome. The second signal triggers the inflammasome formation, resulting in maturation of IL-1 β and IL-18^{98,99}. It has been shown that HSV as well as Varicella-zoster virus (VZV) infection activates inflammasome during early infection^{7,92}. HSV infection transiently induces NLRP3 expression and its association with ASC in human fibroblasts, resulting in IL-1 β maturation. Whether IL-1 β maturation results from IFI16, NLRP3 or both is unclear. Although there is no evidence demonstrating how NLRP3 senses HSV, HSV-1 infection induces oxidative stress and increases the levels of reactive oxygen species (ROS)¹⁰⁰, which is an activator of NLRP3 inflammasome^{98,99}. Additionally, HSV infection activates PKR¹⁰¹, which may contribute to NLRP3 inflammasome activation.

HSV Modulation of IFN Induction

HSV is able to persist despite the active immune defense of the host. It is, therefore, not surprising that they encode an array of gene products to neutralize or evade innate immunity¹². Accordingly, this may prevent viral clearance, which facilitates the

establishment of HSV chronic infection. Several HSV proteins are reported to modulate the induction of innate immunity (Table 1).

Infected cell protein (ICP) 0

ICP0 is an immediate early protein of HSV that inhibits the antiviral action of IFNs¹⁰². ICP0 also inhibits the induction of ISGs early in HSV infection¹⁰³. The RING finger domain of ICP0 is required to inhibit IRF3- and IRF7-mediated activation of ISGs¹⁰⁴. Studies suggest that HSV-1 reduces nuclear IRF3 accumulation, which is dependent on ICP0^{105,106}. Furthermore, ICP0 recruits the activated IRF3 and CBP/p300 to nuclear structures away from the host chromatin during early HSV-1 infection, which suppresses IFN- β and ISG transcription¹⁰⁶. A separate study suggests that cytoplasmic but not nuclear ICP0 is responsible for IRF3 inhibition in HSV-1 infected cells and the proper cellular localization of ICP0 but not the inhibition of IRF3 requires proteasome¹⁰⁷. Intriguingly, ICP0 operates in the nucleus to inhibit IFI16-induced IRF3 signaling in human foreskin fibroblasts⁸¹. ICP0 specifically promotes degradation of IFI16 in the nucleus in a proteasome-dependent manner. Thus, ICP0 interferes with IRF3 signaling at multiple locations inside the cells.

ICP0 is a potent inhibitor of TLR-mediated innate response¹⁰⁸. ICP0 appears to exploit a negative feedback function of ubiquitin-specific-processing protease 7 (USP7), a deubiquitinating enzyme that is able to inhibit NF- κ B and MAPK activation triggered by TLR agonists. USP7 normally resides in nucleus. In the presence of ICP0, however, it interacts with ICP0 and translocates to the cytoplasm where USP7 binds and deubiquitinates TRAF6 and IKK γ , thereby inhibiting NF- κ B and MAPK activation. As a result, TLR-triggered production of inflammatory cytokines and IFN- β is impaired by ICP0. Another group has also observed ICP0 inhibition of TLR2-dependent inflammatory responses and NF- κ B signaling during HSV-1 early infection¹⁰⁹. However, ICP0 specifically targets the adaptor proteins MyD88 and Mal for degradation and the nuclear export function of ICP0 is dispensable for such an effect. It is possible that both mechanisms may operate during HSV infection.

ICP27

ICP27, an immediate early protein, has been recently suggested to be involved in inhibiting HSV-induced production of IFNs and ISGs. The ICP27 deletion mutant HSV-1 is a strong inducer of cytokine production in a murine macrophage cell line¹¹⁰. In line with this, high levels of type I IFNs and inflammatory cytokines are noted in the ICP27 deletion mutant infected macrophages and DCs as compared to wild type HSV-1 infected cells¹¹¹. The ICP27 deletion mutant triggers the activation of NF- κ B and IRF3 more potently than wild type HSV-1, suggesting that ICP27 may dampen NF- κ B and IRF3 signaling. ICP27 is shown to inhibit NF- κ B activation by stabilizing the inhibitor of NF- κ B, I- κ B¹¹². However, the mechanism by which ICP27 affects IRF3 pathway is unknown. Interestingly, other studies report that the ICP27 deletion mutant fails to trigger IRF3-mediated ISG induction in human embryonic lung fibroblasts and to induce IRF3 activation in Sendai virus co-infected human endometrial adenocarcinoma^{104,105}. Therefore, the precise role of ICP27 in modulating innate immunity requires further investigation.

γ _{134.5}

HSV γ _{134.5} is a leaky late protein, but expressed early as well^{113,114,115}. It serves to preclude translational arrest mediated by the double-stranded RNA-dependent protein kinase PKR, thus conferring viral resistance to IFN^{101,116,117}. This is linked to its essential role in HSV pathogenesis^{118,119}. Paradoxically, the γ _{134.5} null mutants with secondary mutations elsewhere in the HSV genome inhibit PKR but still remain attenuated in the mouse model^{120,121,122}. Therefore, HSV γ _{134.5} has an additional function(s), which cannot be

compensated for simply by the inhibition of PKR. In line with this notion, a range of antiviral genes such as IFN- β and certain ISGs is found to be up-regulated, albeit differentially, in the $\gamma_134.5$ null mutant virus-infected cells as opposed to those infected by wild type HSV-1 during early infection¹²³, suggesting that $\gamma_134.5$ interferes with the induction of innate antiviral responses. In screen for HSV-encoded functions, one study has revealed that $\gamma_134.5$ inhibits TBK1 activity¹¹⁵. Notably, the $\gamma_134.5$ null mutant virus stimulates IRF3 phosphorylation and the induction of type I IFN responses whereas wild type HSV-1 inhibits such responses during early infection. Indeed, $\gamma_134.5$ associates with TBK1 and disrupts the interaction between TBK1 and its substrate IRF3, leading to suppression of type I IFN and ISG expression. Importantly, inhibition of TBK1 by $\gamma_134.5$ is independent of $\gamma_134.5$'s function to antagonize PKR and is essential for HSV replication¹²⁴.

HSV $\gamma_134.5$ is also suggested to suppress IKK that activates NF- κ B¹²⁵. In this process, $\gamma_134.5$ recruits protein phosphatase 1 via its carboxyl terminal domain, forming a complex that dephosphorylates IKK α/β . This inactivates IKK α/β and subsequently p65/RelA of NF- κ B, reducing inflammatory cytokine production. Consequently, wild type HSV-1 suppresses DC maturation whereas the $\gamma_134.5$ null mutant stimulates the maturation of DCs^{125,126}. Thus, $\gamma_134.5$ targets TBK1 as well as IKK α/β downstream of TLRs, RLRs, and DNA sensing pathways, which partly explains its role as a virulence factor in HSV infection.

Virion host shutoff protein (vhs)

The vhs of HSV is a ribonuclease that degrades both viral and host mRNA¹²⁷. In HSV infection, vhs interferes with antiviral immunity mediated by IFN, which involves JAK1, STAT2, PKR, and ribonuclease L (RNase L)^{128,129,130,131,132}. In addition, vhs attenuates the induction of type I IFNs and ISGs^{130,131}. In murine embryonic fibroblasts, the vhs deletion mutant virus stimulates the expression of IFN- β as well as IFIT1 as infection progresses. In contrast, wild type virus suppresses such a response. Notably, cells infected with the vhs deletion mutant accumulate more viral RNAs. Therefore, these viral RNAs may act as potent PAMPs which contribute to the observed phenotype¹³¹.

HSV-2 vhs inhibits the induction of IFN- β and ISG56 in human vaginal epithelial cells, which coincides with a selective decrease in the expression of TLR2, TLR3, RIG-I and MDA-5¹³³. Unlike wild type HSV-2, the vhs deletion mutant of HSV-2 causes a robust increase of activated IRF3. When ectopically expressed, HSV-2 vhs inhibits IRF3 activation upon infection with Sendai virus or poly(I:C) stimulation, indicating that HSV-2 vhs exerts its activity without any other HSV proteins¹³³. HSV-1 vhs blocks the production of type I IFNs and inflammatory cytokines mediated by a TLR independent pathway in human and murine DCs, which subsequently results in the suppression of DC maturation¹³⁴. Moreover, HSV-1 vhs is able to block NF- κ B activation which is independent of viral replication but has no effect on IRF3 activation in infected DCs⁴⁹. Thus, while vhs functions as a viral inhibitor of innate immunity, the underlying molecular mechanism is to be defined.

Us3

Us3 is a viral kinase involved in HSV viral gene expression, virion morphogenesis, cytoskeletal rearrangement and evasion of host antiviral response¹³⁵. It has been reported that deletion of Us3 renders HSV sensitive to IFN treatment¹³⁶, albeit this sensitivity is obvious only at a low multiplicity of infection (MOI). In addition, the Us3 deletion mutant HSV-1 stimulates IRF3 activation and increases the mRNA levels of TLR3 and type I IFNs as well as the protein expression of IFN-induced MxA in human monocytic cells¹³⁷. These observations suggest that Us3 interferes with the TLR3 pathway and the subsequent induction of type I IFNs and IFN-induced genes. The mechanism by which Us3 works is unclear. A recent study shows that Us3 suppresses TLR2-mediated NF- κ B signaling and

subsequent cytokine expression early during HSV-1 infection¹³⁸. Us3 appears to modulate NF- κ B signaling by inhibiting TRAF6 ubiquitination, but the direct target of Us3 has not been identified. It is suggested that Us3 facilitates viral evasion of TLR2-induced innate response at or before TRAF6 ubiquitination.

Us11

Us11 is a late gene product of HSV, which inhibits PKR-mediated protein synthesis shutoff^{139,140,141}. Us11 has been also shown to inhibit the 2'-5' oligoadenylate synthetase, which mediates the RNase L antiviral pathway¹⁴². Accordingly, Us11 is required to mediate full resistance of HSV to type I IFN in HSV-infected cells¹⁴³. Evidence shows that wild type HSV-1 infection robustly suppresses Sendai virus-stimulated IFN- β production whereas the Us11 mutant virus moderately relieves this inhibitory effect¹⁴⁴, suggesting that Us11 impedes the viral induction of type I IFN. In HSV-1 infected cells, Us11 interacts with RIG-I and MDA5 in an RNA-independent manner and disrupts the interactions between the adaptor MAVS and these RLRs. Consistently, ectopically expressed Us11 abrogates Sendai virus-induced RLR-mediated activation of IRF3.

HSV Modulation of Inflammasomes

Proinflammatory cytokines IL-1 β and IL-18 play a key role in host protection against HSV-1 infection^{145,146}. A recent study has demonstrated that HSV-1 infection does not induce inflammasome activation in human primary macrophages¹⁴⁷. This is supported by the result that IL-1 β is not proteolytically cleaved and secreted by HSV-1-infected cells compared with the cells that are stimulated with a known inflammasome inducer. This observation suggests that HSV-1 can antagonize the inflammasome pathway. In support of this, HSV-1 infection induces activation and subsequent inhibition of IFI16 and NLRP3 inflammasomes in human foreskin fibroblasts⁷. In the early stage of infection, HSV-1 stimulates the formation of inflammasome complexes involving IFI16, ASC, and NLRP3. As viral infection proceeds, HSV-1 induces degradation of IFI16 and caspase-1 is sequestered in actin clusters in the late stage. Indeed, ICP0 is responsible for IFI16 degradation^{7,81}. On the other hand, HSV-1 disrupts the NLRP3 inflammasome although the underlying mechanism is not known. Thus, HSV-1 has evolved to modulate inflammasome-mediated responses, which may facilitate viral infection.

Concluding Remarks

A prominent feature of HSV is to establish lytic and latent cycles. Despite advances in herpesvirus research, the equilibrium between innate immunity, immunopathology, and viral strategies in HSV chronic infection is not well understood. As the first line of host defenses, the innate immune system has evolved a multi-layered surveillance network to recognize, prevent or contain HSV infections. Therefore, PRRs play a seemingly redundant role, which might be relevant to the magnitude, timing or location of host responses. Several TLR mechanisms have emerged in connection with HSV infections. TLR2 recognizes HSV glycoproteins, which results in a pathological response. On the other hand, TLR2 acts in concert with TLR9 to limit viral replication. Importantly, TLR3 mediates the type I IFN response, which precludes HSV encephalitis in human. Nevertheless, much remains to be learned about HSV and TLRs. Although recognition of HSV is linked to RIG-I and MDA5 the regulatory mechanisms are to be explored. Furthermore, the biological relevance of RLRs in HSV infection *in vivo* remains to be established.

Accumulating evidence supports that cytosolic DNA sensors recognize HSV to induce the type I IFN response. DAI, IFI16, DDX41, cGAS, and Ku70/DNA-PK function via the STING-TBK1 axis whereas RNA PolIII acts via RIG-I. In addition, DHX9 and DHX36

relay signals to IRF7. An unresolved question is how DNA sensors activate downstream signaling in response to HSV. Relevant to this are two issues. First, it is unknown whether additional host factors are required for DNA sensor-mediated recognition of HSV. Second, as most DNA sensors described to date reside primarily in the cytosol, it is obscure how they detect HSV that replicates in the nucleus. Although IFI16 is suggested to function both in the cytosol and nucleus, the role of other DNA sensors needs to be investigated. Another key question is whether and how DNA sensors impact HSV lytic cycle, latency or reactivation. It is noteworthy that innate immune system also detects membrane fusion. Thus, recognition of a biological process by the innate immune system is an area for future investigation.

Facing multiple barriers set by the innate immune system, HSV has developed countermeasures to balance host responses. It is apparent that herpes viruses have co-evolved with the host immune system. HSV proteins including Us3, Us11, vhs, ICP0, ICP27 and $\gamma_134.5$ antagonize innate antiviral responses at multiple sites. As these viral proteins belong to the different kinetic classes, they may exert their activities to counteract the innate antiviral immunity in a temporal and/or spatial manner during HSV infection. Further work is needed to understand how viral proteins work coordinately to establish chronic infection of HSV.

Acknowledgments

The author's work is supported by the grant AI092230 from the National Institute of Allergy and Infectious Diseases.

Abbreviations

AIM2	absent in melanoma 2
Cardif	caspase recruitment domains (CARD) adaptor inducing IFN- β
cGAS	cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase
CNS	central nervous system
DAI	DNA dependent activator of IFN regulatory factor
DC	dendritic cell
DDX	DEAD (Asp-Glu-Ala-Asp) box
DHX	DEAH (Asp-Glu-Ala-His) box
DNA-PK	DNA dependent protein kinase
ERIS	endoplasmic reticulum IFN stimulator
HSV	Herpes Simplex Virus
IFI16	IFN- γ -inducible protein
IFN	interferon
IKK	I- κ B kinase
IPS-1	interferon β promoter stimulator
IRF	interferon regulatory factor
ISG	interferon stimulated gene
I-κB	inhibitor of kappa B

LRRFIP1	leucine-rich repeat flightless-interacting protein 1
MAVS	mitochondrial antiviral-signaling protein
MDA5	melanoma differentiation-associated gene
MITA	mediator of IRF3 activation
MRE11	meiotic recombination 11 homolog A
MyD88	myeloid differentiation primary response protein 88
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NLR	NOD-like receptor
NLRP3	NLR family, pyrin domain containing 3
PAMP	pathogen-associated molecular pattern
PRR	pattern recognition receptor
RIG-I	retinoic acid-inducible gene I
RLR	RIG-I-like receptor
RIP	receptor interacting protein
STING	stimulator of IFN genes
TBK1	TANK-binding kinase 1
TIR	Toll/interleukin-1 receptor
TLR	Toll-like receptor
TRAF	tumor necrosis factor receptor-associated factor
TRIF	TIR domain-containing adaptor inducing IFN- β
VISA	virus-induced signaling adaptor

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Highlights

- Herpes simplex virus (HSV) infection triggers and disarms innate antiviral immunity.
- Multiple receptors recognize molecular patterns of HSV.
- In addition, a component(s) or pathway(s) exists to sense HSV-cell membrane fusion.
- HSV has evolved strategies to neutralize the host responses.

Figure 1.

An outline of innate immunity involved in HSV recognition. In response to HSV infection, host pathogen recognition receptors initiate a complex program that activates transcription factors NF- κ B, IRF3 and IRF7. This leads to the induction of IFN- α/β and inflammatory cytokines including precursors of IL-18 and IL-1 β . TLRs on the cell surface or endosomal membrane recognize HSV and transmit signals via MyD88 or TRIF. RIG-I and MDA-5 detect viral RNA in the cytosol and act via MAVS. DNA sensors detect viral DNA and activate the STING-TBK1-IRF3 axis in the cytosol. In addition, a NLR or DNA sensor mediates inflammasome activation, resulting maturation of IL-1 β and IL-18.

Figure 2.

Cytosolic DNA sensing pathways. A panel of DNA sensors exists to mediate type I IFN induction upon HSV infection. cGAS, DDX41, DNA-PK, and IFI16 signal via the adaptor STING. DAI activates TBK1 and triggers NF-κB via RIP1/RIP3. RNA PolIII transcribes DNA into RNA that serves as a RIG-I ligand. Furthermore, DHX9, DHX36, and Ku70 relay signals to activate IRF7 and IRF1. Although MRE11 serves as a DNA sensor, it does not mediate HSV recognition. LRRFIP1 acts via a β-catenin-dependent pathway but its role in HSV recognition has not been reported. IFI16 assembles inflammasomes in response to HSV infection but AIM2 is not required in HSV-induced inflammasome activation.

Table 1**Modulation of the Induction of Antiviral Immunity by HSV**

HSV Protein	Targeted Response	Mechanism of action	References
ICP0	Type I IFNs and ISGs; Proinflammatory Cytokines; Inflammasome	Sequestration of IRF3 and promoting IRF3 degradation;	106
		Inhibition of IRF3 and IRF7 activity;	104;107
		Targeting IFI16 for degradation;	81;7
		Translocation of USP7 to cytoplasm to target NF- κ B and MAPK pathways by deubiquitinating TRAF6 and IKK γ ;	108
		Target MyD88 and Mal upstream of NF- κ B for degradation	109
γ_1 34.5	Type I IFNs and ISGs; Proinflammatory cytokines; DC maturation	Inhibition of TBK1 activity;	115;124
		Directing protein phosphatase 1 to dephosphorylate IKK β and inhibit NF- κ B activation	125;126
ICP27	Type I IFNs; Proinflammatory cytokines;	Inhibition of NF- κ B and IRF3 activation;	110;112
<i>vhs</i>	Type I and III IFNs; Proinflammatory cytokines;	Degradation of TLR2, TLR3, RIG-I and Mda5;	133
		Inhibition of IRF3 and NF- κ B activation;	49;133
Us3	Type I IFNs and ISGs; Proinflammatory cytokines;	Inhibition of TLR3-mediated IRF3 activation;	137
		Inhibition of TRAF6 ubiquitination and thus NF- κ B activation;	138
Us11	IFN- β	Interference with the interactions of RLRs and MAVS	144