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REVIEW

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Toll-like receptors as novel therapeutic targets for herpes simplex virus infection

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Summary

Seropositivity for HSV reaches more than 70% within the world population, and yet no approved vaccine exists. While HSV1 is responsible for keratitis, encephalitis, and labialis, HSV2 carriers have a high susceptibility to other STD infections, such as HIV. Induction of antiviral innate immune responses upon infection depends on a family of pattern recognition receptors called Toll-like receptors (TLR). TLRs bridge innate and adaptive immunity by sensing virus infection and activating antiviral immune responses. HSV adopts smart tricks to evade innate immunity and can also manipulate TLR signaling to evade the immune system or even confer destructive effects in favor of virus replication. Here, we review mechanisms by which HSV can trick TLR signaling to impair innate immunity. Then, we analyze the role of HSV-mediated molecular cues, in particular, NF-κB signaling, in promoting protective versus destructive effects of TLRs. Finally, TLR-based therapeutic opportunities with the goal of preventing or treating HSV infection will be discussed.

KEYWORDS

herpes simplex virus (HSV) infection, novel therapeutic targets, Toll-like receptors (TLR)

1 | INTRODUCTION

Herpes simplex virus serotypes 1 (HSV1) and 2 (HSV2) frequently cause oral-facial, ocular, or genital mucosa infections.[1] Ocular HSV1 infections mostly affect the cornea, leading to corneal scarring, keratitis, and even blindness. HSV1 infection can also cause encephalitis, which may be fatal.

As one of the most prevalent sexually transmitted infections (STI), the prevalence of genital HSV2 differs (16%-97%) depending on age, sex, ethnicity, culture, geographic location, and other factors. In addition, primary HSV2 infection transmitted to the newborn is associated with high morbidity and mortality.[2] Following initial genital infection, HSV2 forms a life-long latency in the sacral ganglia and occasionally reactivates to establish genital lesions.[3] Moreover, these genital lesions favor acquisition of other STIs, in particular, human immunodeficiency virus type 1 (HIV1).[4] Shedding of HSV2 from the genital tract is recurrent and asymptomatic.[5] Intermittent antiviral therapy can suppress current infections while prophylactic use can prevent further relapses. No vaccines to give protection against HSV have been approved.[6] Nevertheless, there have been some positive clinical achievements. For example, in a recent preclinical study, an HSV2 trivalent subunit vaccine containing glycoproteins C, D, and E (gC2, gD2, and gE2) showed immunogenicity in rhesus macaques and displayed more than 97% efficacy in guinea pigs.[6,7] Also, results from a phase III clinical trial study showed that a recombinant glycoprotein D vaccine, conferred approximately 74% prevention of genital HSV disease in women seronegative for both HSV serotypes.[8]

A family of innate immune receptors, namely, Toll-like receptors (TLR) is responsible for induction of antiviral innate immune responses by recognizing virus infection and inducing a spectrum of signaling pathways, which leads to the production of proinflammatory cytokines, chemokines, and interferons. Moreover, TLRs activate antigen presenting cells (APCs) to work in concert with adaptive immunity for infection eradication and establishment of long-term immunity.[9] There are 10 TLRs in humans numbered consecutively (1-10). Ligands for TLRs are single-stranded RNA (ssRNA) viruses for TLR7/8 and dsRNA viruses for TLR3, CpG DNA for TLR9, envelope glycoproteins for TLR2, lipopolysaccharide (LPS) of gram-negative bacteria for TLR4 and flagellin for TLR5.[10] Other distinct classes of pattern recognition receptors (PRRs), which work with TLRs include RNA helicase retinoic acid-inducible gene (RIG I), the NOD-like receptors (NLRs), RIG-I-like receptors (RLRs)/MDA5, the AIM2 inflammasome, the pyrin and HIN200 domain-containing (PYHIN) protein[11] IFI16 and UNC93B1.[12]

HSV1 infection involves modulation of several TLRs, in particular TLR2/3/9, and the presence or absence of TLR2 is critical to the survival of mice with HSV1 infection.[13] Meanwhile, cytoplasmic recognition of dsRNA by RNA helicases such as RIG I and MDA5 provides another means of recognizing viral nucleic acid.[14] TLR9 and RLRs activate distinctly and/or overlapping innate mechanisms, which leads to efficient viral sensing and production of type I IFNs after HSV infection.[15]

Earlier studies recognized numerous HSV-encoded functions that impede antiviral host immunity including ICP0-mediated suppression of cytokine/interferon response,[16] nonspecific degradation of host mRNA by the virion host shut-off (VHS) RNase,[17] inhibition of PKR by US11 and[18] γ34.5, inhibition of MHC-I peptide loading[19] by ICP47, and modulation of TLRs.[11] In addition, recent data show that HSV1 incorporates a human protein, the DEAD-box ATP-dependent RNA helicase (DDX3X) to stimulate HSV1 gene expression and, consequently, virion assembly without inducing interferon production.[20] Other studies define the contribution of microRNAs in herpes simplex encephalitis (HSE), as it is shown that the 75% to 80% of mice with a deficiency of miR-155 are highly susceptible to HSE after ocular infection with HSV1.[21] Also, miR-H6 encoded from HSV1 genome can engage ICP4 to block HSV1 replication and sustain latency.[22]

Besides activating the innate immune response, TLRs also shape the adaptive immune response toward protective or destructive effects. In response, HSV can manipulate TLR signaling toward avoidance of immune responses or even exploit it for its own benefit. Here, we review mechanisms by which HSV tricks TLR signaling to impair innate immunity, and we also analyze the role of HSV-mediated molecular cues, in particular, NF-κB signaling in promoting protective versus destructive effects of TLRs. Finally, TLR-based therapeutic opportunities with the goal of preventing or treating HSV infection will be discussed.[23]

2 | TLRS: STRUCTURE, LOCALIZATION, AND LIGANDS

TLRs are trans-membrane horseshoe-shaped proteins that identify ligands from pathogenic (viral and microbial products) and commensal organisms, as well as endogenous ligands originating from injured cells.[24] The structure of TLRs consists of three domains: ligand recognition domain at the cell surface or inside the cytoplasm, a single transmembrane domain, and the intra-cytoplasmic TIR domain, which binds to the adaptor proteins.[25]

TLR family members number 10 in human (TLRs 1-10) and 12 in the mouse (TLR1-9 and TLR11-13). While TLR1/2/4/5/6/10 are located extracellularly, TLR3/7/8/9 are located in the cytoplasm (within endosome) and recognize nucleic acids produced during viral infections.[26] Also, glycoproteins are recognized by TLR2, double-stranded RNA (dsRNA) by TLR3, ssRNA by TLR7/8, CpG DNA by TLR9, LPS by TLR4, and flagellin[27] by TLR5.

Upon ligand binding, TLR homodimerization (all TLRs except TLR2) or heterodimerization (TLR2) can be switched on by ligation of the TIR domains of two neighboring TLRs, an event that further promotes conformational changes required for activation of the downstream signaling cascade. Heterodimers of TLR2 with TLR6 or TLR1 can form, where the ligand specificity for each dimer will be different.[1] TLRs may also employ coreceptors for full ligand sensitivity, for example, TLR4 recognition of LPS, requires the cooperation of CD14, MD2, and LPS-binding protein (LBP).[28] Also, intracellular cascades call for binding of extra adaptor proteins including the myeloid differentiation factor 88 (MyD88), the TIR domain-containing adaptor protein inducing interferon-β (TRIF/TICAM), MyD88 adaptor-like protein (Mal/TIRAP), and the TRIF-related adaptor molecule (TRAM). While most TLRs recruit one or two adapters, TLR4 employs all of the four adaptor proteins (Figure 1). Negative regulators of TLR function include the Toll-interacting protein (Tollip), the B cell adaptor for PI3K (BCAP), and IRAK-M.[11]

3 | TLR SIGNALING PATHWAYS

As shown in Figure 1, the TLR adaptor protein MyD88 is central to signaling cascade mediated by TLR1/2/5/6/7/8/9, but it is not needed for TLR3-dependent signal transduction events. MyD88 recruits the serine/threonine IL1R-associated protein 4 and 1 (IRAK4/1 and IRAK1) and activates tumor necrosis factor receptor-associated factor 6 (TRAF6). Then, the signal transduces to TGFβ-activated kinase 1 (TAK1), TAK1-binding proteins 1, 2, or 3 (TAB1/2/3), phosphorylation of IkB kinases (IKKs), and dissociation of inhibitor B (IκBα) from NFκB. Further, NFκB proteins translocate to the nucleus and trigger inflammatory cytokine gene expression in cooperation with the family of mitogen-activated protein kinase (MAPK) and activator protein-1 (AP-1).[29] Signal transduction via TLR7/8/9 also activates MyD88-mediated signaling events through interferon regulatory factor 7 (IRF7), which leads to type I interferon (INFα, INFβ) responses. Both TLR2 and TLR4 utilize a second adapter, TIRAP (Mal) for NF-κB activation. TLR3, which is the main contributor to IFN production, utilizes TRIF instead of MyD88. TRIF signaling through receptor-interacting protein 1 (RIP1) or tank binding protein 1 (TBK1) leads to NF-κB or IRF3 activation, respectively. TLR4 also interacts with TRIF, through the fourth adapter protein, TRAM.[30]

4 | HSV STRUCTURE AND LIFE CYCLE

Nuclear-replicating HSV 1 and 2 belong to the herpes virus family, sharing a similar structure with large double-stranded DNA covered by tegument proteins (Figure 2). The linear and GC-rich genomic DNA contains approximately 80 viral genes, which are organized as unique long (UL) and unique short (US) segments.[31] The nucleocapsid and tegument proteins are wrapped in a glycoprotein-studded lipid envelope, which mediates attachment and entry into target cells. After fusion and entry into the host cell, virus is transported to the nucleus by microtubule transport machinery or endocytosis. Subsequently, virus DNA is released from the capsid into the nucleus to initiate the process of viral gene expression, genome replication, virion assembly, and release of new infectious virus.[27] Three classes of HSV1 genes; immediate early (IE), early, and late are expressed in a sequential manner, and IE genes regulate expression of early genes and late genes. Epithelial or mucosal cells are the primary targets of initial infection after which the virus can form latent infection in sensory ganglia.[32] There is a role for noncoding short RNAs, namely, microRNAs

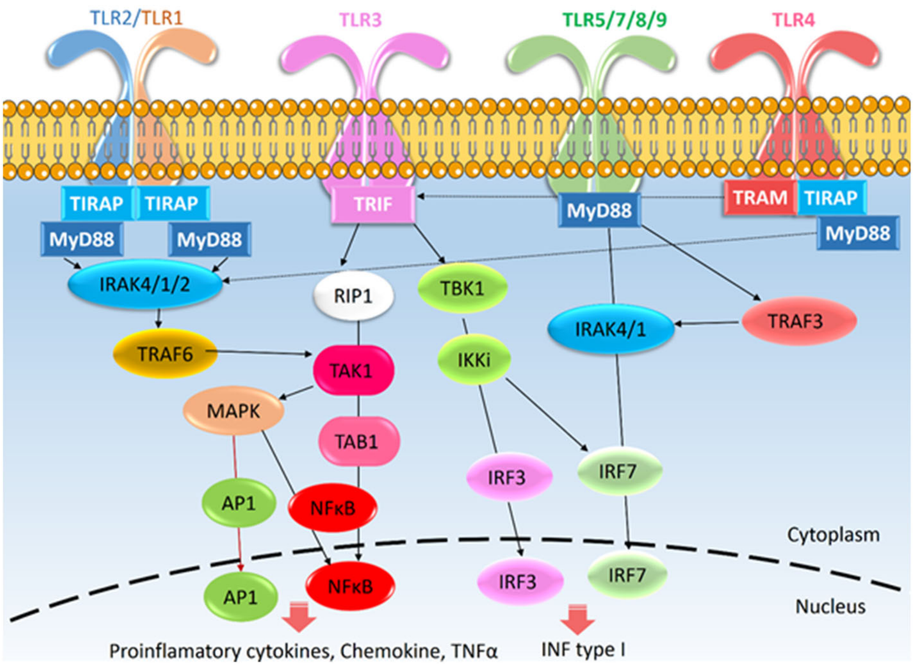

FIGURE 1 Scheme of Toll-like receptor signaling

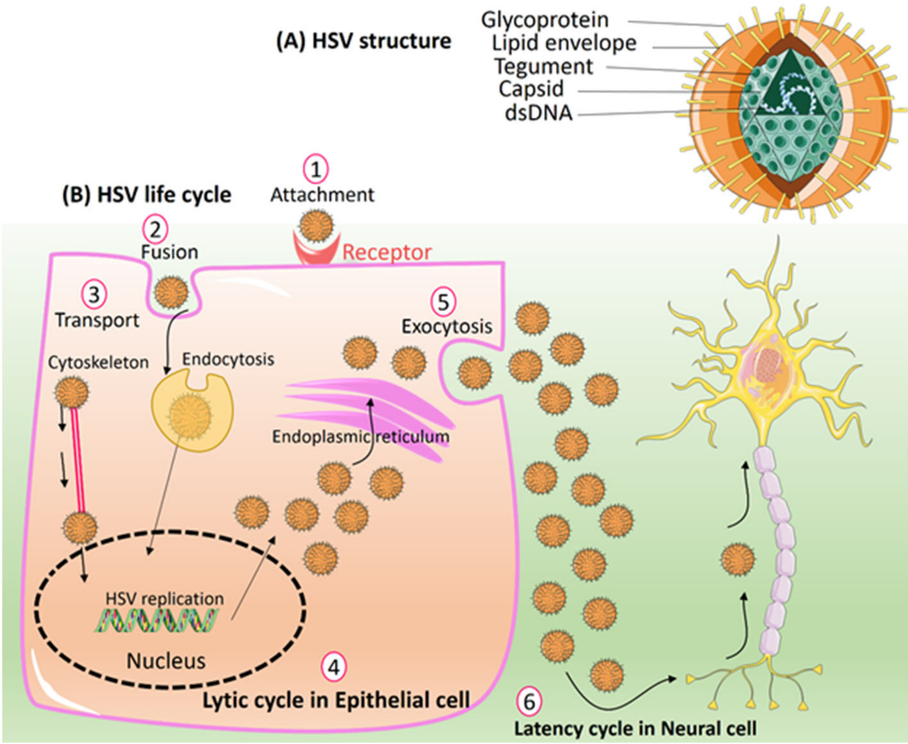

FIGURE 2 Scheme of HSV structure and life cycle

(miRNAs) in HSV latency, because lytic gene expression, IC50 is suppressed by miR-H2, which is completely complementary to ICP0 mRNA.[16] These small sequences are able to regulate the process of gene expression through direct binding of the coding mRNA sequences and further translational impairment.[33-35]

5 | TLR SIGNALING PATHWAYS AND HSV INFECTION

HSV infection elicits a vigorous innate reaction by activating the secretion of a wide panel of chemokines, interferons, and proinflammatory cytokines, involving TLR2, TLR3, and TLR9 or cytosolic RIG I in a Toll-independent manner.[32,36] Several molecular constituents of HSV are capable of activating an innate response, including (a) glycoproteins recognized by TLR2, (b) HSV DNA containing unmethylated CpG motifs detected via TLR9-dependent or non-TLR DNA sensors, and (c) dsRNA and ssRNA recognized by TLR3 and TLR7/8, respectively[37] (Figure 3).

Activation of TLR2 via HSV1-encoded envelope glycoproteins (gB, gC, gD, gE, gH, gL) activates NF-κB via MyD88/TRAF6-dependent signaling pathway[6,8,38] whereas activation of TLR9 by virus DNA (CpG oligodeoxynucleotides (ODN) leads to expression[39] of IFN type I. Likewise, recognition of dsRNA by TLR3 induces type I IFN-mediated antiviral immunity against a number of viral infections. As such, purified HSV2 DNA is shown to trigger IFNα secretion from plasmacytoid dendritic cells (pDCs) and that inhibitory CpG oligonucleotide treatment diminishes HSV-induced IFNα secretion by pDCs in a dose-dependent manner, showing that genomic DNA of a virus can engage TLR9 and result in the secretion of IFNα by pDCs.[39,40] Similarly, HSV-1 can induce IFNβ, via the[41] PYHIN protein IFI16. Moreover, induction of type III interferon (INFλ) contributes to TLR3-mediated HSV1 inhibition in astrocytes and human cervical epithelial cells.[42,43] Dual recognition of HSV by innate Toll system offers an advantage since HSV contains multiple pathogen-associated molecular patterns. As a proof of concept, dendritic cells (DCs) that express multiple TLRs can recognize TLR2 and TLR9 in an orchestrated sequence and can induce IL6 and IL12 secretion from bone marrow-derived DCs.[44] Other studies identified the critical role of TLR2 and TLR9 expressed in trigeminal ganglia for viral control during HSV1 infection.[45] Augmented TLR3/9 gene expression upon stimulation with HSV1 DNA and HSV-anti-HSV IgG complexes results in vigorous IL6 release from infected corneal cells.[46] Importantly, impaired TLR3 and UNC-93B-dependent IFNα/β intrinsic immunity to HSV1 in the CNS, in neurons and oligodendrocytes, explains the pathogenesis of HSE in children.[47]

6 | TLR SIGNALING: FOR OR AGAINST HSV INFECTION

TLR2-mediated cytokine response to HSV1 is detrimental to the host, particularly within the brain. TLR activation is described as a *double-edged sword* since it may either diminish or exacerbate disease, depending on the pathogen and infection site. In this section, we review current knowledge in the context of beneficial versus detrimental effects of HSV-mediated TLR signaling.

In the case of HSV1, the induction of a TLR2-mediated cytokine response in the brain contributes to lethal encephalitis and the death

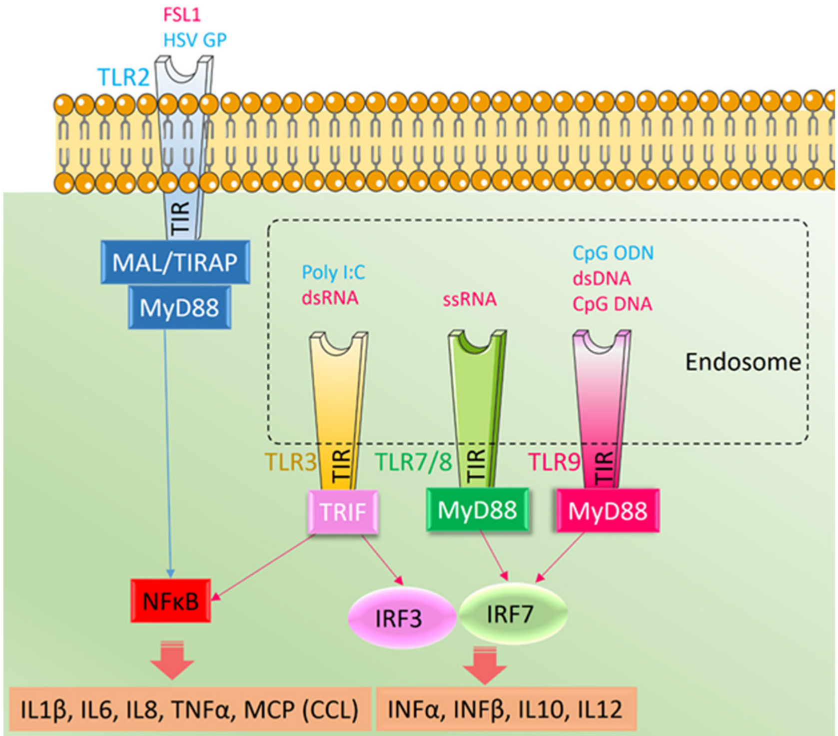

FIGURE 3 Implication of Toll-like receptor signaling during HSV infection. HSV ligands are shown in purple, while theraputic ligands as Toll-like receptor (TLR) modulators are shown in light blue

of the animal.[48] Sepsis syndrome that is seen with HSV infection in neonates can be explained by host responses, as contrary to the predictions, neonates produce more proinflammatory cytokines than adults do. This is in line with the finding that TLR2-deficient mice are more likely to survive HSV1 challenge than wild-type (WT) mice.[49] Another study indicates that HSV-induced expression of inflammatory cytokines by astrocytes, microglial cells, monocytes, and neutrophils is largely facilitated by TLR2 in the central nervous system (CNS). TLR2 induces microglial cell death and apoptosis as a natural defense mechanism to eradicate HSV-infected cells.[50] Besides apoptosis, TLR2 signaling generates ROS and induction of oxidative stress, which facilitates secondary tissue damage during CNS infection and HSE-neurotoxicity. In concordance with this notion, stimulation with HSV1 elevates intracellular ROS and induces more neuronal oxidative damage in WT microglial cell cultures, compared with TLR2-/-microglia, which show a late and lessened ROS formation, reduced p42/p44 ERK and p38 MAPK activation and less cytotoxicity to cultured neurons after viral infection.[51]

In contrast to the destructive effects of TLR2 signaling in HSE, the absence of TLR9 does not impact type I IFN levels, survival rate, or viral replication in the brain following infection, though presence of type I IFNs are protective and absolutely required for survival following intracranial HSV1 infection.[52,53] Surprisingly, other studies describe a protective effect against HSV infection when TLR2/9 works together. As such, TLR2 and TLR9 synergistically fuel innate antiviral events to control HSV infection in the brain,[13] and the low expression of TLR2 and TLR9 in the periphery defines the susceptibility to HSV1 entry into the nervous system.[54]

The effects of TLR3 seem to be protective, as in HSV1-infected cultured mouse neural stem cells (NSCs), HSV-1 infection leads to upregulated expression of TLR3 and the phosphorylation level of IRF3 in the nucleus to induce IFNβ expression. These effects were abrogated after RNAi-mediated blocking of TLR3.[55] Similarly, TLR3 immune deficiency results in HSV2-associated mollaret meningitis.[56] Likewise, TLR3 deficiency renders astrocytes permissive to HSV infection and accelerates CNS infection in mice.[57] Moreover, HSV1 Us3 gene product dampens innate immunity by blocking TLR3 responses in the U937 cultured monocytic cell.[58] Deficiency in TLR-related adaptor molecules, for example, human TRAF3 is another contribution to impaired TLR3 response and susceptibility to HSE.[59]

7 | HSV IMPAIRS TLR SIGNALING AND EVADES IMMUNE CELL RECOGNITION

Herpes viruses usurp different molecular cues to impair host sensing of the pathogen and retard clearance of HSV-infected cells. In recent years, manipulation of TLR signaling by HSV proteins has come to light. It appears that HSV-mediated TLR signaling mainly modulates NF-κB signaling in a way to benefit virus replication while simultaneously endowing suppression of interferon production. It is worth mentioning that the omnipresent NF-κB signaling activates transcription of the key modules of innate feedbacks to viral infection including cytokines, chemokines, adhesion, as well as antiapoptotic proteins. Interestingly, in the case of NF-κB, HSVs modulate NF-κB through numerous viral gene products. That is, HSV not only impairs TLR-mediated NF-κB signaling but can also activate/inhibit NF-κB by its own proteins in a TLR-independent manner to ensure productive infections and immune escape (Table 1).

TABLE 1 Mechanisms employed by HSV to modulate TLR signaling

Mechanisms	TLR Isoforms	Major Findings	References
Antigenic modification	TLR9	Gammaherpesvirus (MHV68) selectively suppress the number of TLR9 immunostimulatory motifs (CpG) through cytosine to thymine conversion.	60
Intrinsic TLR deficiency of host	TLR3	TLR3 immune deficiency leads to HSV2-associated mollaret meningitis.	56
	TLR3	Impaired TLR3 and UNC-93B-dependent IFNα/β intrinsic immunity to HSV1 in the CNS is causative to HSE.	47
	TLR3	TLR3 deficiency renders astrocytes susceptible to HSV infection in CNS.	57
	TLR3	TRAF3 deficiency is relevant to compromised TLR3 response and susceptibility to HSE.	59
	TLR3	Heterozygous TBK1 mutations impair TLR3 immunity and lead to HSE of childhood.	61
	TLR3	NEMO is a key component of NFκB and IRF3-dependent TLR3-mediated immunity to HSV.	62
Remodeling TLR activity	TLR2	Early activation of MyD88-mediated autophagy sustains HSV1 replication in human monocytic THP1 cells.	63
	TLR2	Treatment of TLR2-transfected HEK293T cells with purified HSV1 gB protein activates NFκB reporter and recruits MyD88, TRAF6 but not CD14.	64
	TLR2	HSV US3-mediated TLR2 inhibition occurs at or before TRAF6 ubiquitination.	65
	TLR3	The VHS protein can inhibit DC maturation in absence of TLR-dependent viral recognition.	66
	TLR2	HSV1 break anti-fungal protection by downregulating TLR2 and blocking monocyte activation.	67
	TLR3	HSV1 Us3 inhibit TLR3 responses in cultured monocytic cells.	58
	TLR2	TLR2 signaling activates apoptosis in HSV infected microglia.	50
	TLR9	HSV CpG acts as a TLR9 agonist to stimulate the NFκB activity in HCEn cells for its own replication.	68
	TLR2	HSV ICP0 protein inhibits TLR2-dependent inflammatory responses, NFκB signaling, and immune escape.	69
	TLR2 TLR4	HSV ICP0 recruits USP7 to modulate TLR-mediated innate response	70
	TLR2	HSV U_L37 protein contains a TRAF6-binding domain and activates NFκB through direct recruiting TRAF6 adaptor protein.	71
	TLR9	The Us2 protein of HSV2 modulates NFκB activation by targeting TAK1.	72
	TLR2	HSV induces neural oxidative damage of microglial cell via TLR2.	51

Recently, a screen of the US regions of HSV2 identified the gene product of US2 to positively modulate NF-κB signaling and cytokine production via ligation[72] to TAK1. Conversely, HSV immediate early protein ICP0 interaction with the USP7 (HAUSP) elicits opposite effects on TLR-induced NF-κB signaling. USP7 encodes deubiquitination of IKKγ and TRAF6, which operate downregulation of TLR dependent-NF-κB and subsequent inflammatory mediators. These data pinpoint the negative regulatory role of USP7 in Toll signaling, and HSV ICP0 seizes this potential to counteract innate responses during HSV infection.[70] Likewise, HSV UL37 tegument protein can induce NF-κB without engaging TLR2. The cellular transfection of UL37 was associated with endogenous expression of IL8 gene and subsequent IκB degradation. This activation required TRAF6, and surprisingly, UL37 appears to contain a TRAF6-binding domain.[71]

Other studies have discovered that TLR9-dependent pathways are harnessed by HSV. In this regard, result from one study verified that corneal endothelial (HCEn) cells expressed abundant intracellular level of TLR9 and that the TLR9 ODN, provoked the NF-κB activity in these cells, comparable with HSV1 infection, which also stimulated NF-κB and NF-κB-related inflammatory cytokines, including IP10 (CCL5), CXCL10, monocyte chemoattractant protein-2 (MCP2 known as CCL8), macrophage migration inhibitory factor (MIF), MCP4 (CCL13), MDC (CCL22), MIP3α (CCL20), IL5, TARC (CCL17), and MCP1 (CCL2). Blocking the activity of TLR9 not only significantly reduced the levels of these cytokines but it also inhibited viral replication in HCEn cells, which was restored by a simultaneous NF-κB activation. HCEn cells ignite transcriptional activation of inflammatory actions in response to HSV1 infection including NF-κB, the CCAAT-enhancer-binding proteins (C/EBP), cyclic AMP response element (CRE), plus a series of TLR9-dependent inflammatory cytokines. Alternatively, HSV1 usurps the TLR9-NFκB axis for virus replication.[68]

Recent research illuminates how NF-κB activity is synchronized by HSV to favor immune escape during the very early phase of viral infection. HSV infected cell protein 27 (HSV1 ICP27), an IE protein of HSV1, represses rather than activates NF-κB activity by ligating to IkBα, and Daxx, blocking phosphorylation and ubiquitination of IkBα and thus stabilizing IkBα.[73]

HSV can block activation of innate immunity by direct suppression of TLR signaling. As such, HSV1 dysregulates antifungal defenses, which downregulates TLR2 and avoids monocyte activation.[67] Likewise, HSV US3 tegument protein inhibits TLR2 signaling at or before TRAF6 ubiquitination.[65] Another mechanism for HSV ICP0 inhibitory potential on TLR2-driven NF-κB signaling is via degradation of adaptor proteins and IRF3. ICP0 alone can counteract TLR2-evolved responses to either viral or nonviral ligand upstream of p65 and at or downstream of MyD88. ICP0 expression alone can also dampen the MyD88 and TIRAP levels.[69] HSV ICP0 is also shown to recruit USP7 to suppress NF-κB and JNK activation and TLR2/TLR4 mediated innate response.[70] Also, HSV1 Us3 can interfere with the TLR3 sensing of HSV-related ligands and subsequent induction of type I IFN inducible MxA protein levels by type I IFN in monocytic cells.[58] Likewise, OASL1 deficiency increases antiviral immunity toward genital HSV2 infection by improving type I interferon expression of IRF7. Oasl1(-/-) mice displayed superior survival rates, suppressed virus replication, enhanced production of type I IFNs, and cytotoxic T cell responses including IFNγ production than WT mice following intravaginal HSV2 infection.[74]

8 | TLR-BASED THERAPEUTIC OPPORTUNITIES FOR HSV

HSV has favorable biological features that can be employed to fight viral infection. Two tactics can be envisioned as (a) developing HSV-based vectors (amplicons) and (b) TLR modulators using either HSV amplicons or TLR ligands with agonistic, antagonistic, or adjuvant capabilities (Figure 4).

8.1 | Herpes simplex virus-based vectors (amplicons)

Natural neurotropism has led to the development of HSV-based vectors for neuronal gene delivery. Now, versatile and high titer HSV-based gene vectors are designed and implemented in the therapeutic and prophylactic settings to attack infectious diseases and cancer,[75-78] possibly improving the efficiency of gene targeted molecules like naked siRNA[79] or even serving for new generation genome editing tools like CRISPR/Cas.[80]

As a versatile gene transfer platform, the replication-defective HSV1 amplicon has gained significance because of its amenability to genetic manipulation, its widespread cellular tropism, extensive transgene capacity, and minimal immunogenicity.[81] There are two types of vectors: amplicon vectors, which are plasmids wrapped into HSV particles using a helper virus and replication-defective viruses, which are nontoxic forms of virus due to deletion of viral genes.[82] Numerous studies have revealed a significant role of innate immune responses induced by virus vectors in activation of inflammatory responses and the control of transgenic expression.[83-85] Thanks to the HSV amplicons, we can study innate molecular cues stimulated by the entry of HSV1 particles without expression of the viral gene.[31] In this respect, HSV1 amplicon vectors as gene transfer agents and potential to carry costimulatory genes such as CD80 (B7.1) or CD154 (CD40L) have shown promising results in immunotherapy of chronic lymphocytic leukemia (CLL). The results of one study noted that, although the transduction efficacy of two vectors were similar, surprisingly, HSV amplicon vectors that were packaged using a helper virus (H+-HSV) or without it (HF-HSV) showed opposing effects on CLL B cells. Adjuvant immunostimulatory and potent anti-CLL response was associated with the HF-HSV, whereas H+-HSV displayed an immunosuppressive activity, which inhibited the development of

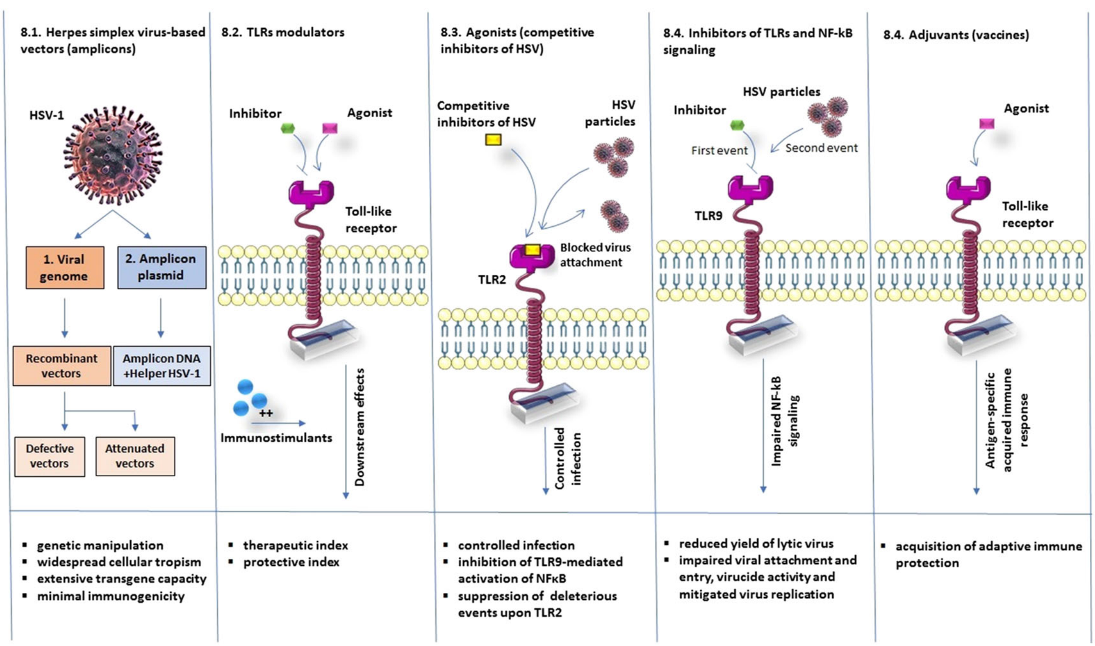

FIGURE 4 Toll-like receptor (TLR)-based therapeutic opportunities for HSV

TABLE 2 TLRs based HSV drugs

TLR Isoform	HSV Serotype	Mechanism	Compound	Major Findings	References
TLR4	HSV2	Adjuvant	gD2-AS04 (aluminum hydroxide and 3-O-deacylated monophosphoryl lipid A (MPL)	Completed	NCT00224484
TLR4	HSV2	Adjuvant	G103 (gD, UL19, and UL25 recombinant proteins) + synthetic TLR4 agonist glucopyranosyl lipid A (GLA)	HSV2 subunit vaccine induces GLA-dependent CD4 and CD8 T cell responses and protective immunity in mice and Guinea pigs.	[90]
TLR3	HSV1	Agonist	Type III INF (INFλ)	Poly I:C-induced interferonγ is required for TLR3-mediated HSV1 inhibition in astrocytes.	[42]
TLR3 TLR9	HSV1	Agonist	IL-29, IL-28A	Inhibit HSV1 replication in neuronal cells through interaction with IL10Rβ and activation of IRF7, IFNα, and the key IFNα stimulated antiviral genes MxA, OAS-1, PKR, and ISG56.	[91]
TLR7	HSV2	Agonist	Topical SMIP-7.7	Protects against genital herpes simplex virus type-2 disease in the Guinea pig.	[92]
TLR3 TLR8	HSV1	Agonist	Poly-I:C ssRNA	Activation of TLRs and IFNα/β expression inhibits HSV1 infection in human neuronal cells.	[93]
TLR3 and RIGI	HSV2	Agonist	INFλ	The topical treatment of genital mucosa with poly I:C could protect mice from genital HSV2 infection.	[43]
TLR7/8	HSV2	Agonist	Imidazoquinolines (imiquimod and resiquimod (R-848)	Topical resiquimod 0.01% gel decreases HSV2 genital shedding in human.	[94]
TLR9	HSV1	Inhibitor	Oligonucleotide containing five adjacent guanosine residues (G-ODN)	Reduced NFκB activity in ARPE19 and Vero cells.	[95]
TLR4 (TBK1)	HSV1	Agonist	Defective virus (Gamma134.5-/-) + CD11(+) DCs	Engineered HSV-mediated activation of TBK1 is crucial for DC maturation and inducing protective immunity.	[96]
TLR3	HSV1	Agonist	Amplicon vector	Upregulation of TLR3, IRF7, and IFN through IRF3/7 activate the innate response in human fibroblasts.	[87]
TLR 2/6	HSV2	Agonist	FSL-1, a bacterial-derived diacylated lipopeptide	Bacterial-derived TLR2/6 agonist FSL1 induce significant resistance to HSV2 infection in mice/human vaginal EC cultures.	[97]
TLR2	HSV1	Agonist	Low-molecular-weight mannogalactofucans (LMMGFs)	LMMGFs enhance TLR2 expression, antagonize viral adsorption via TLR2 in Vero cells.	[98]
TLR1, TLR4, TLR6, TLR7, TLR8, TLR9, TLR10 TLR2, TLR3, TLR5	HSV2	Agonist Inhibitor	Longdanxiegan traditional Chinese medicine (LDXGFG)	TLR1/4/6/7/8/9/10 significantly decrease while, TLR2/3/5 increase in both DCs, and Langerhans cells. The LDXGFG corrected the abnormal expression of TLR pathway genes in genital herpes and recurrent genital herpes Guinea pigs.	[99]
TLR9	HSV2	Adjuvant + agonist	gD and gB neutralizing antibodies (nabs) + tegument protein UL40 + the agonist CpG oligodeoxynucleotide formulated in a squalene-based oil-in-water emulsion	Induce a robust HSV2-specific cell-mediated immune response, protect against symptomatic disease, and reduce the latent viral reservoir.	[100]

(Continues)

TABLE 2 (Continued)

TLR Isoform	HSV Serotype	Mechanism	Compound	Major Findings	References
TLR3 TLR4 TLR9	HSV1	Agonist	Polyinosinic:Polycytidylic acid (poly I:C) synthetic dsRNA analog Lipopolysaccharide (LPS) ODN	TLR3 stimulation by poly I:C 24 h before infection resulted in a significantly lower virus load, 94% survival of mice and reinforces a natural innate immune mechanism of neuroprotection in a mouse model of HSE.	101
TLR3 TLR9 TLR9	HSV1	Agonist Agonist Antagonist	Polyinosinic: Polycytidylic acid; PIC 1585, 1826, or 2395 ODNs ODN 2088	The best timing for agonist and antagonist immunization was determined before and postinfection, respectively, in a mouse model of HSE.	102

antitumor T-cell immunity.[86] Also, HSV1-based amplicon vectors have identified the presence of activation pathways for the virus, which work independently of TLR and rely on IRF3/7 activation. Infection of human fibroblasts with amplicons confers antiviral response via significant upregulation of TLR3, IRF7, and IFN-stimulated genes (ISGs), rendering HSV-cells immune to virus infection and vesicular stomatitis virus.[87]

8.2 | TLR modulators

Efficient immune responses require close interaction between the innate and adaptive immunity and TLRs play a fundamental role by linking these two systems together. The innate immune system not only reacts promptly to environmental insult or microbial infection but also instructs and activates APCs to produce cytokines for T cell polarization toward a proper effector phenotype.[88] Through appropriate antigen presentation, only mature DCs will be able to stimulate differentiation of naive T cells into effector T cells. The pattern of cytokines induced by the TLR engagement will determine the type of effector T cells.[89] Thus, TLR seems an ideal target to treat/avoid/protect a wide spectrum of immune-related disease/infections.

TLR-based HSV therapy with natural/synthetic compounds or gene therapy using amplicon vectors entails three modalities including agonists, inhibitors (antagonist), and adjuvant therapy to achieve a therapeutic/protective index (Table 2).

8.3 | Agonists (competitive inhibitors of HSV)

Agonists act as competitive inhibitors for HSV to bind to TLRs. Poly I:C, ssRNA, virus glycoproteins, and attenuated virus (containing mutant genes) are examples of ligands, which have been successfully employed in preclinical and clinical settings.[103] Agonist therapy can control infection at the very early stage since they can block virus attachment to the cell surface via TLR2 or they can block TLR9-mediated activation of NFκB signaling by virus. Additionally, since agonists block the interaction of virus with TLRs, they may suppress deleterious effects of TLR2 in response to HSV as seen in HSE cases.

The antiviral activity of low-molecular-weight mannogalactofucans (LMMGFs) illustrates its potential as a potent TLR2 agonist. LMMGFs enhance TLR2 mRNA expression and stimulate the phosphorylation of Akt and JNK in Vero cells. LMMGFs inhibit viral entry and also exhibit inhibitory activity directly against viral particles. These results clearly demonstrated that LMMGFs use TLR2 as their receptor, preventing HSV1 infection on the host cell surface and antagonizing viral adsorption via TLR2 pathway activation in Vero cells.[98] Also, defective viruses can be employed as agonists with vaccine potential. Recombinant HSV1 with a mutation in the gamma134.5 protein, a virulence factor, can stimulate DC maturation ($CD11^+$) via activation of TBK1 and sequential phosphorylation of IRF3 and p65/RelA. Immunizations with the gamma134.5 induce immune responses and protect mice against lethal challenge by WT virus. Additionally, mutant virus-activated DCs elicit immunity upon adoptive transfer.[96]

8.4 | Inhibitors of TLRs and NF-kB signaling

Inhibitors of TLRs significantly inhibit virus replication by interfering with NF-κB signaling at an early stage of virus infection. For example, treatment with a five adjacent guanosine residues (G-ODN) at a concentration of 10 to 20 μM 2 hours before infection inhibited TLR9 signaling, NF-κB activity and substantially reduced the yield of lytic virus (90%) in herpes-susceptible cells. Also, the TLR9 inhibitory effect of CpG oligonucleotide was associated with downregulation of crucial immediate early HSV proteins, impaired viral attachment and entry, virucide activity and mitigated virus replication.[95] Equally, an additional study using both agonists (TLR 3/9) and inhibitors (TLR9) of TLRs demonstrated an increased survival rate of mice when agonists of TLR3 polyinosinic: polycytidylic acid (PIC) and TLR9 (type B ODN 1826) were administered intranasally prior to HSV1 infection. In contrast, the results of antagonist therapy were positive when TLR9 inhibitor ODN 2088 was given after viral infection. Interestingly, post-treatment with PIC conferred opposite effects and was translated into an aggravated HSE compared with the control. These observations were possibly due to the stimulatory effect of agonist therapy on early production of type I IFN to reduce viral load in the brain whereas the encouraging effects of antagonist therapy on HSE survival rate was related to the diminished expression of inflammatory mediators such as CCL5, TNFα, and IL6 post infection.[102]

8.5 | Adjuvants (vaccines)

Given the potential of TLR agonists to bring innate and adaptive immunity together by activating APCs such as immature DCs to mature DCs and conferring effective Th1(CD4+) and Th2 (CD8+) and INFγ responses, TLR agonists can indeed make good adjuvants as well.[104] In one clinical report of a phase III clinical trial, gD2-AS04 containing HSV2 glycoprotein D2 and aluminum hydroxide and 3-O-deacylated monophosphoryl lipid A (MPL) was successful as a TLR4-based vaccine.[105] Correspondingly, resiquimod, a TLR7/8 agonist, is capable of inducing cytokine production to stimulate an antigen-specific Th1-acquired immune response, which adjusts HSV infection in vivo. Also, resiquimod 0.01% gel reduced human anogenital HSV2 mucosal reactivation.[94] A complete list of other TLR-based immune modulators of HSV infection, and their mechanism of action is provided in Table 2.

9 | CONCLUDING REMARKS AND FUTURE DIRECTIONS

With regards to their potential to delicately fine-tune the immune response, TLRs are excellent candidates for eliminating viral infections. Nevertheless, precautions should be taken into consideration, and knowledge about the role of molecular cues, which determine protective versus detrimental effects of TLRs can help to optimize TLR therapy. Thus, extra attention should be devoted to new drugs that can encourage substantial and longstanding immunity, while concurrently easing unwanted effects. An understanding of how precise constituents of HSV induce and/or prevent innate immunity would open the door for rational design of gene therapy vectors and TLR modulators explicitly personalized for specific clinical applications. Natural/synthetic TLR modulators combined with HSV amplicon vectors would be advantageous to boost innate immunity for vaccination means, while their potential to inhibit TLR-NF-*k*B signaling is optional to avoid the unwelcome inflammatory responses as described in HSE cases.

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CONFLICT OF INTEREST

The authors have no competing interest.

ABBREVIATIONS

APCs	antigen presenting cells
BCAP	B-cell adaptor for phosphoinositide 3-kinase
CCL5	Chemokine (C-C motif) ligand 5
CD11	cluster of differentiation 11
CNS	central nervous system
CRISPR/Cas	Clustered regularly interspaced short palindromic repeats/caspase
Daxx	Death-associated protein 6
DDX3X	DEAD-box ATP-dependent RNA helicase
DNA	Deoxyribonucleic acid
dsRNA	Double-stranded RNA
ERK	extracellular signal–regulated kinases
FDA	Food and Drug Administration
gC2	HSV-2 glycoproteins C
gD2	HSV-2 glycoproteins D
gE2	HSV-2 glycoproteins E
G-ODN	guanosine-rich oligodeoxynucleotides
HAUSP	HSV immediate early protein ICP0 interaction with the USP7
HCEn cells	human corneal endothelial cells
HIV	human immunodeficiency virus
HSE	herpes simplex encephalitis
HSV	Herpes simplex virus
ICP0	Infected Cell Polypeptide 0
ICP4	infected cell polypeptide 4
ICP47	Infected cell protein 47
IE	immediate early
IFNs	Interferons
IKKs	I kappa B kinases
IL6	Interleukin 6
INFα	Interferon alpha
INFβ	Interferon beta
INFλ	interferon lambda
IRAK	Interleukin-1 receptor-associated kinase
IRF7	Interferon regulatory factor 7
IκBα	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
LBP	LPS-binding protein
LMMGFs	Low-molecular-weight mannogalactofucans
LPS	lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MCP2	monocyte chemoattractant protein-2
MDA5	Melanoma Differentiation-Associated protein 5
MHC-I	major histocompatibility complex class I
miRNA	microRNAs
MyD88	myeloid differentiation factor 88
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NLRs	NOD-like receptors
NSCs	neural stem cells
OASL1	2′-5′-oligoadenylate synthetase-like protein

ODN	oligodeoxynucleotides
pDCs	plasmacytoid dendritic cells
PIC	polycytidylic acid
PKR	protein kinase R
PRRs	pattern recognition receptors
PYHIN	Pyrin and HIN200 domain-containing
RIP1	Receptor-interacting protein 1
RLRs	RIG-I-like receptors
SRI	sexually transmitted infections
ssRNA	single-stranded RNA
STD	sexually transmitted diseases
TAB1/2/3	TAK1-binding proteins 1, 2 or 3
TAK1	TGFβ-activated kinase 1
TBK1	Tank binding protein 1
TICAM	TIR domain-containing adaptor molecule
TLR	Toll-like receptors
Tollip	Toll-interacting protein
TRAF6	tumor necrosis factor receptor-associated factor 6
TRAM	TRIF-related adaptor molecule
TRIF	TIR domain-containing adaptor protein inducing interferon-β
UNC93B1	Unc-93 homolog B1
Us	unique short
VHS	Virion host shut-off
WT	wild-type

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