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Immune Evasion by Herpes Simplex Viruses

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Abstract

Infection with herpes simplex viruses type 1 (HSV-1) and type 2 (HSV-2) is extremely frequent in the human population, as well as recurrent reactivations due to lifelong infection. Infection and persistence of HSVs within healthy individuals likely results as a consequence of numerous molecular determinants evolved by these pathogens to escape both immediate and long-term host antiviral mechanisms. Indeed, HSVs harbor an arsenal of proteins that confer them stealth by negatively modulating immune function. Consequently, these viruses perpetuate within the host, altogether silently shedding onto other individuals. In this chapter, we discuss HSV determinants that interfere with cellular antiviral factors, as well as viral determinants that hamper innate and adaptive immune components intended to control such microbes. The identification of HSV evasion molecules that modulate the immune system, as well as the understanding of their mechanisms of action, should facilitate the design of novel prophylactic and therapeutic strategies to overcome infection and disease elicited by these viruses. This chapter is intended to provide an overview of the evasion mechanisms evolved by herpes simplex viruses to escape numerous host antiviral mediators.

Keywords: immune evasion, innate immunity, adaptive immunity, antiviral response

1. Introduction

Herpes simplex viruses (HSVs, HSV-1 and HSV-2) are extremely prevalent in the human population with virtually half of the world inhabitants infected with HSV-1 [1] and nearly 500 million with HSV-2 [2]. Novel infections with HSVs are estimated at a rate of dozens of millions individuals per year [3]. Importantly, the prevalence of HSV infection significantly varies



© 2016 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. depending on the geographical location of individuals, sex, and ethnicity [3–7]. While primary HSV-1 infection is well known for its pathological effects in the oro-facial area, where it mainly produces lesions in the mouth, it is also responsible for most cases of infectious blindness in developed countries [8–21]. On the other hand, HSV-2 is widely recognized as an important contributor to neonatal encephalitis and, most importantly, the main cause of genital ulcers in the world [8–21]. Nevertheless, despite this latter association between HSV-2 and genital infection, HSV-1 is at present the main cause of primary genital infection [9, 12]. This apparent paradox may be explained by the fact that HSV-2 recurs significantly more frequently in the genitalia than HSV-1, while the opposite occurs for the oro-facial area [22]. Such differences may be accounted by disparities in the capacity of each of these viruses to establish latency in the sacral and trigeminal ganglia [23], although another study proposes that this is not the case [24]. Regardless of differences in neuron infection or reactivation capacity from these sites, overall HSV-2 is isolated more frequently than HSV-1 in the genitalia during the lifespan of an individual [23]. Importantly, HSV-2 is considered at present a meaningful contributor to the fueling of human immunodeficiency virus (HIV) infection in the world, and is discussed in detail below [22, 25–27].

Besides viral encephalitis in neonates, as well as oro-facial and genital lesions, HSV-1 and HSV-2 are also responsible for numerous other diseases in humans, such as adult encephalitis, herpetic keratitis, conjunctivitis, and skin lesions, within many other clinical manifestations [4]. The variety of pathologies produced by HSVs and tissues affected may be due, at least partially, to the wide distribution of their receptors, which are virtually present on all cells of the body [28]. Noteworthy, such clinical outcomes can occur indistinguishably both in immunocompetent and immunocompromised individuals and are likely a result of the evolution and selection of HSV determinants that interfere with early antiviral cellular mechanisms, innate- and adaptive-immune components. Noteworthy, HSV genomes encode numerous gene products (at least 70), which likely warrants these microbes a collection of proteins with immune evasion properties [29]. Below, we discuss several of these viral determinants, as well as how they interfere with host antiviral processes.

2. Herpes simplex viruses escape early antiviral responses

2.1. Interference with host pathogen recognition receptors

Upon encounter with foreign molecules or danger elements, host immune and nonimmune cells may sense such stimuli and initiate intracellular-activating signaling pathways that lead to their alertness and that of surrounding cells. Importantly, complex host organisms have evolved as specialized receptors for recognizing these microbial elements or self-induced danger molecules during abnormal processes elicited by these microbes. Such receptors are usually termed pattern recognition receptors (PRRs) [30]. PRRs can recognize pathogen-associated molecular patterns (PAMPs), which consist on a diverse collection of biomolecules derived from microbial elements, such as proteins, lipids, carbohydrates, or particular arrangements or sequences of nucleic acids, within others [31]. Alternatively, these receptors

can also detect danger signals released by host cells undergoing stress circumstances, such as those that might be elicited by virus replication. These latter danger signals are termed damage-associated molecular patterns (DAMPs) [32]. Upon engaging PAMPs and DAMPs, PRRs elicit intracellular signals that result in the transcription and translation of antiviral genes, as well as the expression of soluble and membrane-bound molecules. Timely and robust detection of PAMPs and DAMPs by the host after viral infection can lead to effective microbe control and promote the establishment of protective immunity [31, 33].

Upon exposure to HSVs, the main host cells susceptible to infection are likely live epithelial cells. These cells are largely present at the interphase with the exterior world and abundantly present in the mucosae and to a lesser extent, in microscopic skin lesions. As most nonimmune cells in the organism, these cells express the main HSV receptor, nectin-1 [28]. After attaching and binding to their receptors, the membranes of these viruses undergo a fusion process with that of the host cell to release the viral capsid and surrounding tegument proteins within the cytoplasm [34]. While the tegument proteins remain in the cytoplasm, where they exert numerous cellular modulatory effects, the capsid associates to microtubules and travels to the outer nuclear membrane, where it binds to host nuclear pore proteins and releases the viral DNA into the nucleus [35]. It has been described that at this stage host molecular sensors can sense HSV-2 determinants (Table 1). Interferon-gamma inducible-16 (IFI-16) detects the HSV genome and subsequently induce IL-6 and IFN- α production in primary vaginal epithelial cells [36-38]. On the other hand, the cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS), a recently described DNA sensor, has also been reported to detect HSV-derived nucleic acids and lead to IFN- α and IFN- β secretion by both immune cells, such as macrophages and dendritic cells (DCs), and nonimmune cells, such as fibroblasts [39]. Importantly, animals that lack cGAS are vulnerable to HSV, while functional cGAS leads to T-cell activation and antibody production by B cells [40]. Although HSVs seem to be unable to interfere with cGAS sensing, other herpesviruses (gammaherpesviruses) have been recently described to encode viral determinants that impair the function of this molecule, namely, ORF52 of Kaposi's sarcoma herpesvirus [41]. Interestingly, a recent study suggests that IFI16 and cGAS work cooperatively to sense HSV, as silencing one or both proteins significantly decreases virus detection. More specifically, cGAS was shown to directly interact with IFI-16 in fibroblasts and to promote the stability of the latter [39]. Noteworthy, both sensors IFI-16 and cGAS signal intracellularly through interferon regulatory factor-3 (IRF3) and again silencing either sensor inhibits the activation of IRF3 in response to HSV DNA [42]. Further, the importance of IFI-16 in limiting HSV infection has been recently shown in vivo. Knocking down IFI-16 led to the loss of IFN- α production, as well as reduced viral control in the corneal epithelium [38]. While the mechanism by which IFI-16 recognizes HSV DNA remains somewhat unclear, a recent study using chromatin immunoprecipitation (ChIP) found that IFI-16 binds to HSV promoter sequences and that reducing the levels of IFI-16 expression resulted in host proteins binding to these elements, ultimately favoring viral gene transcription [43].

Host	Viral determinant involved	Outcome	Mechanism	References
IFI-16	Nuclear virus DNA	HSV sensed. IL-6, IFN- α secretion	IFI-16 binds to viral promoters	[36–38, 43]
cGAS	Nuclear virus DNA	HSV sensed. IFN- α , IFN- β secretion	cGAS binds directly to B-DNA	[39]
DAI	Cytosolic virus DNA	HSV sensed. IL-6, IFN-β secretion	DAI binds directly to B-DNA	[36]
MDA5	vhs	Unable to sense viral nucleic acids	vhs protein reduces host protein expression	[44, 45]
RIG-1	vhs	Unable to sense viral nucleic acids	vhs protein reduces host protein expression	[44, 45]
TLR-9	Cytosolic virus DNA	HSV sensed. IL-6, IL-12, type-I IFN secretion	Undetermined	[36, 46]
PKR	$\gamma 34.5$ and $U_{\rm S}11$	Impaired viral dsRNA recognition	Viral proteins block eIF2- α phosphorylation	[53–57]
TLR7	Undetermined	HSV sensed	Undetermined	[58]
TLR3	Virus or virus-induced host nucleic acids	HSV sensed. IL-6, TNF- α secretion	TLR-3 signals through IRF3	[63–65]
ανβ3	ICP0	Inhibited detection of viral proteins	ICP0 blocks type-I IFN transcription and virus targeting to degradation	[74–76]
TLR2	dUTPase	HSV sensed. IL-6, IL-8, IL-10, IL-12, and TNF- α secretion	Undetermined	[77]
Inflamm– asome	ICP0	ICP0 reduces IL-1β secretion	Inflammasome directed to proteasome degradation in timely manner	[32, 43]

IFI-16 (gamma-interferon-inducible protein); cGAS (cyclic GMP-AMP synthase); DAI (DNA-dependent activator of Interferon-regulatory factor); MDA5 (melanoma differentiation-associated protein 5); vhs (virion host shutoff protein); RIG-1 (retinoic acid-inducible gene 1); TLR-2, -7, -9 (Toll-like receptor-2, -7 and -9); PKR (protein kinase RNA-activated); $\alpha\nu\beta3$ (integrin alphaVbeta3); $\gamma34.5$ (late gene gamma 34.5, ICP34.5); U_s11 (short unique region 11); IRF3 (interferon regulatory factor 3); ICP0 (infected cell protein 0); IL-1β, -6, -8, -10, -12 (interleukin-1β, -6, -8, -10, and, -12); IFN-α/β (interferon alpha and beta); TNF-α (tumor necrosis factor-alpha).

Table 1. HSV evasion of host virus sensing.

Another host DNA sensor capable of detecting HSV genetic material is DNA-dependent activator of interferon (DAI), which is expressed in primary vaginal epithelial cells and leads to cytokine expression by these cells, such as IL-6 and IFN- β after virus exposure (**Table 1**) [36]. Surprisingly, DAI is expressed in the cytoplasm, suggesting that HSV DNA likely escapes or leaks from the nucleus, or capsids into the cytoplasm where it reaches this sensor.

Other nucleic acid detectors intended to perceive microbe-derived genetic material are retinoic acid-inducible gene-1 (RIG-I) and melanoma differentiation-associated protein-5 (MDA5) (**Table 1**) [44]. Unlike the other DNA sensors discussed above, the functions of RIG-I and MDA5 are hampered by HSV, namely, by the viral protein designated virion host shutoff protein (vhs). The vhs has been shown to specifically reduce the expression RIG-I and MDA5, as a mechanism to interfere with downstream signaling events carried out by these detectors, which are intended to alert neighboring and immune cells when viral elements are present (**Table 1**) [45]. Similar to DAI, RIG-I and MDA5 are also present in the cytoplasm of cells, which indicates that HSV DNA likely reaches this compartment during the infectious cycle [36].

Another PRR that also recognizes viral DNA is Toll-like receptor-9 (TLR9), which is mainly known for its role in sensing bacterial-derived nucleic acids, namely, CpG-oligodeoxynucleotides (CpG ODNs). TLR9, which is expressed both by immune and nonimmune cells, has been shown to detect HSV elements and produce IL-6, IL-12, and type-I IFN, within others (**Table 1**) [36, 46]. Although TLR9 is capable of sensing HSV, its function seems nonessential for animal survival upon viral challenge. Indeed, TLR9 knockout mice survive central nervous system (CNS) infection, although they do display increased viral loads in the brain, when compared to wild-type mice [47]. Remarkably, animals treated with TLR9 agonists, such as CpG ODNs previous to infection, display significantly reduced viral loads and inflammatory cytokines in the brain (CCL2, IL-6, and CCL5) [48]. A similar protective effect has been observed for CpG ODNs in mice that were treated locally with such stimulators and then challenged in the genitalia with HSV [49–52]. These results suggest that engaging TLR9 receptors, or promoting their signaling pathways may be a promising strategy for preventing HSV burden in the host.

Although the genomes of HSVs are composed of DNA, these viruses produce viral RNA molecules during their infectious cycles that are generated as a consequence of transcription. These RNA molecules are then processed into mRNAs and miRNAs that may form tridimensional structures, which could be recognized by host sensors. One such sensor is host protein kinase R (PKR), which can detect double-stranded RNA molecules and mediate downstream signaling events that lead to limited virus replication by favoring NF-kB activation and cytokine release, while altogether inhibiting protein synthesis through the phosphorylation of the host translation initiation factor 2-alpha (eIF2 α), which ultimately can promote cell death (apoptosis) [53]. To date, numerous studies have demonstrated that HSV can indeed interfere with PKR function both *in vitro* and *in vivo* in such a way to promote their infectious cycles (**Table 1**) [54, 55]. Furthermore, interference with the capacity of PKR to phosphorylate eIF2 α has been shown to be mediated by the HSV proteins γ 34.5 and U_s11, which allows viral protein synthesis to occur efficiently within infected cells [56, 57].

Another nucleic acid-sensing molecule capable of recognizing double-stranded RNA species produced during viral infection is TLR7, although to date this particular receptor has not been described to sense any particular form of nucleic acid generated during HSV infection (**Table 1**). Nevertheless, some studies report that the application of TLR7 agonists, such as imiquimod to experimental animals can significantly decrease HSV infection and disease after virus challenge [58]. Such findings have led to the assessment of imiquimod as a therapeutic approach to treat HSV infection in humans, particularly for combating HSV isolates that are

resistant to acyclovir, which are commonly found in immunocompromised patients [59–61]. For example, a recent report described successful treatment of hypertrophic genital herpes in a HIV-positive patient after using 5% imiquimod applied in a topical manner after repeated failure to resolve the symptoms in the patient with oral and intravenous antivirals [62]. Although the results obtained till date on this type of approach have been promising, the mechanism of action of imiquimod over HSV remains unclear, as both interferon-dependent and interferon-independent mechanisms seem to play favorable roles against viral infection, which may further be mediated by processes that are independent of TLR engagement [60].

Lastly, another host nucleic acid sensor is TLR3, which is mainly known to recognize doublestranded RNA [63]. Importantly, TLR3 has been reported to play relevant roles in HSV disease, although its participation during infection has mainly been inferred by its deficiency (Table 1). For instance, TLR3^{-/-} mice display severe HSV burden within the CNS after infection, which is thought to be mediated by astrocyte infection. Indeed, the expression of TLR3 in such cells increases the control of HSV infection early after virus entry into the CNS, seemingly by inducing type-I IFN responses [64]. Such interferon response would be mediated by TLR3induced NF-kB activation in astrocytes and a posterior increase in the expression of IL-6 and TNF- α , which likely play antiviral functions in this tissue [65]. A relevant role for TLR3 in humans was initially proposed for infants, but has now extended onto adults thanks to recent studies performed on individuals that carry mutations in this receptor that negatively modulate its function. For instance, individuals harboring mutations in TLR3 have been reported to display a history of HSV encephalitis [66-69]. Additionally, a direct relationship between downstream TLR3 signaling, TLR3 defects, and virus burden in astrocytes has been shown with ex vivo differentiated neurons, astrocytes, and oligodendrocytes obtained from patients that display TLR3 deficiencies. Cell cultures derived from these individuals and infected with HSV show reduced virus control in vitro, as compared to cells obtained from controls, which secreted more interferon [70]. Furthermore, HSV-susceptible individuals have also been reported to display mutations in proteins that are involved in the downstream signaling of TLR3, such as in IRF3 [69, 71, 72]. As with TLR9 and TLR7, agonists for TLR3 such as polyI:C have also been shown to reduce viral burden when applied in the genitalia or intraperitoneally previous to a genital challenge with HSV [73]. Overall, these results highlight an important role for TLR3 in HSV encephalitis, altogether proposing potential new treatment alternatives for reducing HSV burden in the brain and other tissues.

While HSV-derived nucleic acids are perceived by numerous host sensors in infected cells, relatively few HSV proteins have been described to be detected by the host (**Table 1**). Integrin $\alpha\nu\beta3$, which has seldom been recognized as a PRR was recently described as a sensor for HSV, which is negatively modulated by these viruses. Furthermore, the HSV protein ICP0 has been proposed to be responsible for blocking the signaling events triggered by integrin $\alpha\nu\beta3$ within infected cells, which otherwise would lead to NF-kB activation, type-I IFN transcription, and the direction of virus particles to cholesterol-rich microdomains that are targeted for degradation [74–76].

An HSV protein that is successfully detected by host sensors is the viral dUTPase, which has been shown to be sensed by TLR2 in DCs and leads to IL-6, IL-8, IL-10, IL-12, and TNF- α

secretion (**Table 1**) [77]. Interestingly, TLR2 has also been reported to recognize other HSV elements, with partial modulation by TLR9 [78]. Noteworthy, experiments performed with TLR2^{-/-} knockout mice showed that these animals displayed increased survival rates, as compared to wild-type animals after challenge with HSV, which suggests a potentially negative role for this receptor in disease severity. Yet the knockout animals had similar levels of viral loads in their tissues, as control animals [79]. Remarkably, microglia cells obtained from TLR2^{-/-} mice have been shown to produce reduced levels of reactive oxygen species (ROS) after HSV infection, when compared to control cultures, which might result in decreased cellular oxidative toxicity to neurons and positively impact on their viability [80].

The inflammasome is a host multiprotein complex harboring the cytoplasmatic sensors NLRP3, AIM2, and IFI-16, which has been described to sense HSV constituents, although its activation is negatively modulated during infection (**Table 1**) [32]. While IFI-16 and NLRP3 are activated early after HSV infection with consequent IL-1 β release, at later time points IFI-16 has been reported to be directed to the proteasome by the viral protein ICP0 [32, 43]. This observation implies that the overall function of this sensor complex is likely hampered by HSV and thus limited at properly alerting other cells of an ongoing viral infection.

Taken together, HSVs seem to be sensed by host cells mainly at the nucleic acid level rather than protein level. This observation is quite surprising considering that HSVs encode numerous gene products within their genomes (>70 ORFs) and at least 11 surface glycoproteins. This stealth attribute might be explained within others by the ability of these viruses to interfere with downstream signaling events mediated by PRRs, as discussed in detail below. Additionally, their apparent invisibility might also be a consequence of the viruses' capacity to interfere with host translation of mRNA transcripts that encode soluble and membrane-bound mediators required for cell alertness after infection and also communicating infection onto other cells. Indeed, the HSV-1 and HSV-2 vhs proteins efficiently inhibit the translation of host mRNAs by promoting their degradation directly through their ribonuclease activity [81]. Importantly, increased degradation of host transcripts over viral mRNAs would be mediated by the spatialtemporal regulation of vhs expression in infected cells, as vhs proteins are delivered together with the tegument immediately after infection, then poorly expressed during viral replication and then abundantly produced immediately before virus packaging and exit [82]. More recently, vhs proteins have been reported to interfere with stress granule formation within infected cells, thus counteracting host antiviral stress responses that are usually elicited early after infection [83].

2.2. Negative modulation of interferon pathways

An effective mechanism by which host cells restrict viral replication is due to interferons, soluble proteins, that induce antiviral responses both in cells that secrete these mediators as well as neighboring cells [84]. While type-I interferons (IFN- α , IFN- β , and IFN- ϵ , within others) are usually secreted early after microbe infection by diverse cell types, type-II interferons (IFN- γ) are secreted by specific subsets of immune cells at later stages of infection. On the other hand, type-III interferons (IFN- λ 1, IFN- λ 2, and IFN- λ 3) have similar effects and kinetics than type-I IFNs, although they are mostly restricted to epithelial cells [63, 85–89]. While type-I and

type-III IFNs induce multiple antiviral effects in most host cells, type-II IFNs play more regulatory roles, mainly in immune cells.

Target host molecule	Viral determinant	Outcome	Mechanism	References
IRF3 function	ICP0	Inhibits type-I IFN expression	ICP0 RING finger motif inhibits IRF3-mediated transcription of interferon stimulating genes	[90, 91]
	U _s 3	Decreases IFN-β production	Viral Ser/Thr kinase activity hyperphosphorylates IRF3, blocking its dimerization and nuclear translocation	[92]
	U _L 36	Inhibits IFN-β transcription	U_L 36 de-ubiquitinates TRAF3, which inhibits stimuli-induced dimerization of IRF3	[94]
IRF3, NF-kB	VP16	Inhibits IFN-β expression	Inhibits NF-kB activation and blocks the recruitment of IRF3 co-activator CBP	[93]
STAT-1 function	ICP27	Neutralizes expression of IFN-I	Interferes with nuclear accumulation of STAT-1, impairing with the activity of this transcription factor	[96]
STAT-2 function	Undetermined, vhs (partially)	Interferes with IFN-I signaling	Partially attributed to vhs-mediated reduction of transcription factor activity	[97, 98]
Jak-1 function	Undetermined, vhs (partially)	Interferes with IFN-I signaling	Partially attributed to vhs-mediated reduction of transcription factor activity	[97, 98]
IFN-ε, IFN-α	Undetermined	Reduces viral dissemination and reactivation	Activates TLR signaling through unknown viral agonist	[99–102]
IL-29 function	Undetermined	Induces IFN-β expression. Prevents keratinocyte and neural infection	Unknown viral antigen activation of TLR3 and Jak-STAT signaling	[104]
IL-28A function	Undetermined	Prevents neural infection	Unknown viral antigen. TLR-mediated activation of IRF7	[105]

IRF3 (interferon regulatory factor 3); NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells); STAT-1, -2 (signal transducer and activator of transcription-1, -2); Jak-1 (Janus kinase 1); IFN- ε , IFN- α (interferon-epsilon, -alpha); IL-28A, -29 (interleukin-28A, -29); U_L36 (long unique region 36); VP16 (viral protein 16); ICP27 (infected cell protein 27); TRAF3 (TNF receptor-associated factor 3); CBP (CREB-binding protein).

Table 2. HSV interference with host intracellular signaling.

HSVs encode numerous virulence factors that negatively modulate the induction of host interferon responses and their effects, some of which were briefly discussed above (**Table 2**).

For instance, HSVs interfere with PRR-mediated intracellular signaling events that otherwise would lead to the transcription of IFN-I. Such effect has been reported to be mediated by HSV proteins such as ICP0, which interferes with IRF3 to block the transcription of target genes, namely, type-I IFNs [90, 91]. On the other hand, the HSV Ser/Thr kinase U_s3 has also been shown to interfere with signaling mediated by this transcription factor by carrying out its hyperphosphorylation, which blocks its dimerization, nuclear translocation, and hampers IFN-β production [92]. Additionally, the tegument protein VP16 has also been described to abrogate IFN-β expression by inhibiting IRF3 and NF-kB activation, specifically by impairing the recruitment of the coactivator CBP (CREB binding protein) and not necessarily through a mechanism that affects IRF3 dimerization, nuclear translocation, or its DNA binding activity [93]. Finally, IFN-β transcription has also been reported to be inhibited by the viral ubiquitinspecific protease U_L36, which de-ubiquitinates TRAF3 (TNF receptor-associated factor-3) and consequently inhibits stimuli-induced IRF3 dimerization [94]. Interference with signaling events that lead to type-I IFN secretion has also been evidenced in vivo by the observation that only reduced amounts of IFN- α and IFN- β are produced in the genital tract after HSV infection [90, 95]. Negative modulation of the interferon pathway is summarized in Table 2.

Although small amounts of type-I IFNs are produced during HSV infection, the effects of these meager amounts of interferons are neutralized by HSVs, thanks to the activity of the viral protein ICP27, which interferes with STAT signaling (signal transducer and activator of transcription), which is located downstream of IFN-I receptors. Indeed, HSV ICP27 interfere with nuclear accumulation of STAT-1 and impair the function of this transcription factor [96]. Additionally, other transducers of IFN-I signaling, such as STAT-2 and JAK1 (Janus Kinase), have also been reported to be reduced in HSV-infected cells and experiments with mutant viruses suggest that these effects would be mediated, at least partially by virally encoded vhs [97]. Additional viral and nonviral determinants released by HSV-infected cells have been suggested to interfere with IFN-I signaling, although their nature has not been fully characterized [98]. Although IFN- ε signals through similar receptors than IFN- α and IFN- β , this recently described mediator is constitutively expressed by epithelial cells in the genitalia and would likely play a role against HSV burden [99-101]. However, the mechanism by which IFN-ε would limit HSV infection remains to be determined. Consistent with an important role for type-IIFNs in response to HSV infection, treatments with TLR agonists, such as imiquimod, poly(I:C), or CpG-ODNs, discussed above all induce strong interferon responses [51, 52, 58– 61, 73]. Additionally, application of topical IFN- α has been shown to significantly reduce viral dissemination, as well as the frequency of viral recurrences in HSV-infected patients that manifest frequent genital viral reactivations [102]. The role of IFN-I in HSV infection is also evidenced in experiments assessing mice that lack the receptor for this molecule (IFNAR1 and IFNAR2c), which were inoculated in the footpads with the virus. These animals displayed reduced HSV control and systemic infection that affected multiple organs, although the disease was nonlethal [103].

At present, several studies seem to have identified a favorable role for type-II IFNs in HSV infection, as discussed in the following sections. Yet surprisingly, one study that evaluated

infection in mice that had the IFN-II receptor deleted (IFNGR1 and IFNGR2) showed that these animals had comparable levels of virus than wild-type controls [103].

Unlike type-I IFNs, relatively few reports have documented a role for type-III IFNs in HSV infection. One such study has reported that IFN- λ 1 (IL-29) induces the expression of several antiviral proteins in human keratinocytes. Furthermore, administration of this cytokine, previous to HSV infection induced IFN- β and prevented keratinocyte infection upon HSV challenge. Notably, the effect of this interferon depended on TLR3 expression, which was upregulated, and JAK-STAT activation [104]. Additionally, IFN- λ 1 and IFN- λ 2 (IL-28A) have also been reported to suppress HSV infection in human neurons (**Table 2**). Again, IFN-III was shown to induce TLR expression and elicit TLR-mediated antiviral pathways that involved IRF7 [105]. Noteworthy, the secretion of type-III IFNs in the vaginal mucosa has been suggested to be mainly mediated by DCs, although this has not been evaluated yet in the context of an HSV infection [106].

Taken together, HSVs have evolved multiple mechanisms to interfere with host interferon responses, from IFN transcription to IFN signaling. These evasion mechanisms, largely redundant, highlight the importance of this type of response in limiting HSV infection. Indeed, novel therapeutic strategies seem to share in common the induction of type-I IFNs, which should facilitate the identification of novel formulations that provide beneficial effects against these viruses.

2.3. HSV modulation of cell viability

Viruses utilize cells as substrates for replication and require, within others their translation machinery for synthesizing their proteins. Maximization of virion production is favored by extended cell survival and thus viruses have evolved molecular mechanisms to inhibit cell apoptosis. As discussed above, interference with programed cell death is achieved in part by the blockage of virus detection, but also thanks to viral determinants that directly hamper this cellular antiviral function. Cellular apoptosis can be mediated by two major pathways triggered either by intrinsic or extrinsic stimuli [107]. While the intrinsic pathway can be initiated by intracellular events that alter the redox state of the cells, damage the host DNA, or compromise mitochondrial integrity, within others, the extrinsic pathway can be elicited by the engagement of surface receptors, such as Fas [107]. HSVs have evolved molecular determinants that block both, intrinsic and extrinsic apoptosis signaling pathways in infected cells (Table 3). For instance, inhibition of apoptosis has been described to be mediated by the viral proteins U_s3, U_s5, and U_s12, each with unique inhibitory effects over the viability of the infected cells, either confronted or not to cytotoxic T cells [108]. More specifically, the HSV protein U_s3 has recently been described to mediate its antiapoptotic effects in epithelial cells, by interacting with programmed cell death protein 4 (PDCD4) and retaining it within the nucleus of infected cells [109]. HSV ICP10PK and U₁14 would also harbor antiapoptotic effects in neurons and epithelial cells, although the mechanisms mediating these effects remain unknown [110, 111].

On the other hand, inhibition of apoptosis in HSV-infected cells by the extrinsic pathway has been suggested to occur by the sequestering of Fas ligand, which consequently would hamper Fas/FasL (CD95/CD95L) engagement, and thus block the capacity of T cells to mediate the

killing of target cells [112]. Additionally, a recent study found that although some HSVinfected cells express Fas on their surface, HSVs can block Fas-mediated apoptosis by a mechanism that is independent of viral activation of NF-kB, as this transcription factor could be detected within the nucleus of infected cells [113]. Consistent with altered Fas/FasL function in HSV-infected cells, therapeutic application of soluble Fas ligand has been reported to ameliorate acute and recurrent herpetic stromal keratitis (HSK) in mice, by reducing inflammatory infiltration into the eye and decreasing eye neovascularization in primary and recurrent forms of HSK [114]. Interestingly, HSV glycoproteins gJ and gD have been proposed to mediate, at least partially the inhibition of Fas-mediated apoptosis [115, 116]. Paradoxically, gJ induces ROS within cells, which could trigger intrinsic pro-apoptotic stimuli. On the other hand, HSVs have been recently described to be able to suppress necroptosis in human cells as a mechanism to extend cell viability [117]. The viral proteins ICP6 and ICP10 have been recently described as the viral determinants that block necroptosis elicited by TNF in human cells [117]. The effects of HSV determinants over cell viability are summarized in **Table 3**.

Cell type	Viral determinant	Outcome	Mechanism	References
Epithelial	U _s 3	Prevents apoptosis	Acts retaining PDCD4 within the nucleus	[108, 109]
Fibroblast	U _s 5	Prevents apoptosis	Unclear. Inhibition of caspase 3 activation	[108]
Fibroblast	U _s 12	Prevents physiologic and CTL-induced apoptosis	Unclear. Inhibition of caspase 3 activation	[108]
Epithelial and neuronal	ICP10PK and U _L 14	Prevents apoptosis	Unknown	[110, 111]
Neuronal	gD and gJ	Prevents apoptosis	Inhibits Fas-mediated pathway	[115, 116]
Neuronal	LAT	Prevents cold shock- induced apoptosis	Maintains high levels of phosphorylated AKT in cells	[125]
Epithelial	ICP6 and ICP10PK	Prevents necroptosis	Blocks cell death elicited by TNF	[117]
Dendritic cell	Undetermined	Induces cell death-related processes	Unclear	[118–120]
Natural killer	Undetermined	Induces apoptosis	Induction of Fas/FasL through infected macrophage expression	[121]
Fibroblast	ICP6	Induces necroptosis	Interacts with host RIP-3	[122]

 U_s 3, 5, 12 (short unique region 3, 5, 12); ICP10PK (ICP10 serine-threonine protein kinase); U_L 14 (long unique region 14); gD, gJ (glycoprotein D, J); LAT (latency-associated transcript); AKT (protein kinase B); ICP6, 10 (infected cell protein 6, 10); CTL (cytotoxic T lymphocyte); PDCD4 (programmed cell death protein 4); RIP-3 (receptor-interacting protein kinase 3); TNF- α (tumor necrosis factor-alpha).

Table 3. HSV modulation of cell death.

Contrarily to the observations discussed above, other studies have reported proapoptotic effects for HSV proteins, as well as pronecrotic effects. For instance, infection of murine and human dendritic cells with HSV induces apoptosis, but after the virus has negatively modulated some of their properties ([118–120] and below). Additionally, HSV has also been described to induce apoptosis in natural killer cell (NK cells) upon their interaction with infected macrophages that express Fas/FasL [121]. On the other hand, HSVs have been described to induce necroptosis in mouse fibroblasts through the direct interaction of viral ICP6 with host RIP3 (receptor-interacting kinase 3) [122]. Importantly, an HSV virus with ICP6 deleted was unable to produce necrosis in HSV-infected cells and RIP3^{-/-} mice displayed compromised control of HSV pathogenesis and replication [123].

Importantly, several reports have described that neuron infection with HSV does not lead to cell death, but rather extends their life. Indeed, lower levels of caspase-3 transcripts have been found in HSV-positive, rather than HSV-negative trigeminal ganglia and neurons [124]. Furthermore, HSV determinants such as the latency-associated transcript (LAT), which is mainly expressed within infected neurons has been reported to protect cells from cold-shock-induced apoptosis by maintaining high levels of phosphorylated protein kinase AKT within the cells [125].

Taken together, these studies highlight unique properties of HSV in their capacity to modulate cell viability in a cell-specific manner. While the viability of nonimmune cells that serve as a substrate for virus replication is extended by antiapoptotic viral determinants, immune cells are targeted for death in such a way to evade the immune system. Noteworthy, neurons which act as reservoirs for the virus are maintained viable during infection.

2.4. Soluble mediators secreted shortly after HSV infection

Despite the capacity of HSV to limit the cell's capacity to sense viral components or transduce activating signals after virus detection, which otherwise could lead to optimal antiviral responses, cells infected with HSV nevertheless secrete numerous cytokines upon infection. Secretion of these cytokines might be promoted by host sensors that effectively detect HSV determinants or alternatively might result from virus-oriented immune modulation intended to promote infection and persistence [32]. Thus, to date it is unclear whether soluble mediators secreted by HSV-infected cells either contributes to virus control and spread or promotes the virus' life cycle within the host.

Signaling events that lead to the secretion of soluble mediators after microbe or danger signal detection is frequently mediated by the nuclear factor NF-kB, which translocates from the cytoplasm to the nucleus to promote gene transcription [126]. Because of the importance of NF-kB in this process, HSV encode several determinants to dampen the activity of this transcription factor. For instance, the HSV U_s3 kinase has been reported to hyperphosphorylate NF-kB (p65) and impairs its translocation to the nucleus, interfering with IL-8 secretion [127]. On the other hand, the HSV U_L42 protein, which encodes for a DNA polymerase processivity factor, also binds to p65/RelA and to p50/NF-kB1 (NF-kB1 forms) to negatively modulate their migration into the nucleus after stimulation with TNF- α [128]. HSV ICP0 has also been described to inhibit NF-kB activation mediated by TNF- α , by interacting similarly with p65/

RelA and p50/NF-kB1 [129]. HSV VP16 similarly has been shown to interfere with NF-kB activation in human endothelial kidney cells [93]. Paradoxically, other studies have proposed that HSV infection can induce persistent NF-kB nuclear translocation, although without concomitant transactivation activity and in epithelial cells from the retina (retinoblastoma) [130, 131]. Importantly, blocking NF-kB nuclear translocation in these cells significantly reduced virus yield. Altogether, these studies demonstrate significant modulation of this important transcription factor by HSV determinants, which may result in different cellular outcomes depending on the cell type infected.

Although HSV negatively modulates NF-kB activation, HSV-infected cells can produce numerous soluble mediators. For instance, primary endometrial genital epithelial cells infected with HSV produce CCL2, IL-8, IL-6, and TNF- α [132]. On the other hand, samples from the cervical mucus of women infected with HSV show elevated amounts of CXCL9 [133]. The latter chemokine together with CXCL10 have been shown to participate in antiviral responses against HSV in CNS infection in the mouse model by recruiting NK and cytotoxic T cells to the infected tissue [134]. Other chemokines, such as CCL2, which are promoted by HSV infection, may play positive roles against the virus in ocular infection, as shown in mice; CCL2 knockout mice displayed significant viral infection and reduced inflammatory monocyte recruitment into the affected tissue [135]. On the contrary, blocking specific chemokines such as CXCL2 which is released by monocytes in response to HSV is thought to bring neutrophils into the infection site, which would promote unwanted damaging responses to the host, particularly neurons [136].

HSV also induces IL-6 in numerous cell types after infection, such as microglia, mast cells, and dendritic cells. Importantly, this cytokine shows protective effects in microglia, which seems to be mediated by STAT3; however, the details of the processes that converge toward its protective effects remain unresolved [137, 138]. Mast cells secrete IL-6 early after HSV infection, as well as TNF- α . Interestingly, these soluble mediators are not promoted by HSV directly in this case, but by soluble molecules secreted by keratinocytes infected with HSV [139]. Importantly, animals that lack IL-6 or TNF- α succumb to death after HSV infection, which indicates that these soluble molecules play positive antiviral effects for the host [139]. Nevertheless, other studies propose that this latter cytokine might play a negative role for the host, as treating animals with an anti-TNF- α antibody in combination with the antiviral valacyclovir significantly ameliorated the prognosis of HSV encephalitis [140]. On the other hand, before DCs are killed by HSV, these cells secrete IL-6 and numerous other cytokines [119].

Noteworthy, HSV has also been described to induce the secretion of cytokines and chemokines that could favor host infection by other sexually transmitted microbes, such as the human immunodeficiency virus [141]. Accumulating evidence indicates that infection with HSV-2 can increase host susceptibility up to fourfold to acquiring HIV [142–145]. Additionally, coinfection with these pathogens augments the shedding of both viruses, likely worsening patient prognosis. Importantly, similar findings have been observed in the mouse model with HIV/HSV coinfections [146]. Such increase in the susceptibility of acquiring HIV in HSV-infected individuals would be mediated by numerous factors, such as the increased recruitment, to the infection site of cells that are targeted by HIV [147–149]. Furthermore, cells infected

with HSV secrete cytokines that reactivate latent HIV from infected cells [119, 150] and may augment the expression of surface ligands that promote HIV infection [151, 152]. Finally, HSV infection can downmodulate the expression of molecules that favor the neutralization and destruction of HIV [153].

A soluble mediator frequently associated with virus control and clearance is IFN- γ . This innate and adaptive immune cytokine is recurrently associated with increased protection against HSV in numerous HSV infection models and considered an important mediator in the mechanism of action of different prophylactic formulations [154–157]. Importantly, in the absence of IFN- γ , T cells directed against HSV secrete alternative cytokines that are known to possess antiviral functions, yet they are not protective against genital infection [158]. Thus, IFN- γ seemingly plays an important role in eliciting protective immunity against HSV.

Taken together, HSVs elicit the secretion of cytokines and chemokines both by immune and nonimmune cells. Yet, whether these soluble mediators play favorable roles for the host or these viruses remains somewhat unknown and requires further examination. To date, only type-I IFNs and IFN- γ seem to play evident favorable roles against HSV.

3. Herpes simplex viruses interfere with innate immunity

3.1. Interference with complement function

Complement is an acellular component of innate immunity that recognizes foreign elements and subsequently undergoes a series of controlled molecular chain reactions that either culminate with the establishment of a protein pore-forming complex that attacks bilipid membranes or induce receptor-mediated engulfment by cells [159]. Importantly, formation of the pore complex can be promoted either directly by the recognition of microbial molecular patterns on the surface of the virus by complement components, or induced by the Fc portion of antibodies that bind to foreign elements.

To counteract the effect of the complement, HSVs utilize glycoprotein gC, which binds to C3b and blocks its activity by impairing antibody-induced complement activation (**Table 4**) [160, 161]. Inhibition of C3 impairs complement-mediated virus inactivation and the lysis of virus-infected cells. Furthermore, gC also binds to complement component C5 to block its down-stream activities, such as immune cell chemoattraction and membrane attack complex formation (**Table 4**) [162, 163].

HSVs have also evolved molecular determinants that bind to complement components required for antibody-mediated complement activation, as discussed below. Thus, by interfering with complement components HSVs increase their viability in the mucosae and sera of infected patients, which favors the infection of target cells.

Innate immune	Viral	Outcome	Mechanism	References
process altered	determinant			
Complement	gC	Inhibits antibody-mediated complement activation	Acts as a receptor for complement component C3b	[160, 161]
Complement	gC	Impairs chemoattraction and membrane attack complex formation	Binds to complement component C5 hampering its catalytic activity and inhibiting downstream events	[162, 163]
Natural killer	gD	Suppresses NK degranulation and cell-mediated lysis of infected cells	CD112 downregulation leading to reduced DNAM-1 activity	[168]
Natural killer	Undetermined	Decreases NK activation	Reduces the surface expression of MICA, ULBP1, ULBP2 and ULBP3	[169–171]
Natural killer	Undetermined	Induces cell apoptosis	Induces Fas/FasL interactions through infected macrophages	[121]
Natural killer T cells	U _s 3	Inhibition of antigen presentation to NKT cells	Phosphorylation of KIF3A produces CD1d downregulation in infected cells	[175–177]

gC, gD (glycoprotein C, D); U_s3 (short unique region 3); C3b, C5 (complement component 3b, 5); NK (natural killer cell); CD112 (nectin-2); DNAM-1 (DNAX accessory molecule-1); MICA (MHC class I polypeptide-related sequence A); ULBP1, 2, and 3 (UL16 binding protein 1, 2 and 3); KIF3A (kinesin family member 3); CD1d (antigen-presenting glycoprotein CD1d).

Table 4. Evasion of innate immunity.

3.2. Negative modulation of NK and NKT function

Besides complement, innate immunity is also composed of numerous cells, such as macrophages, neutrophils, mast cells, basophils, eosinophils, and innate lymphoid cells (ILCs), within others. Importantly, HSV modulates the function of some of these cells, notably NK (Table 4). NK cells usually play important roles in eliminating infected cells that have lost class I major histocompatibility complex molecules (MHC-I) on their surface, because of microbe interference. Although NK cells can directly sense HSV through TLR2 and have been reported to be activated by plasmacytoid dendritic cells that have contacted HSV, their function is nevertheless dampened by the virus [164-167]. Indeed, gD has been shown to suppress DNAM-1-dependent NK-cell-mediated lysis of HSV-infected cells [168]. Furthermore, HSVs can dampen the surface expression of the NK-activating ligand MICA (MHC class I polypeptide-related sequence A) in infected cells, by retaining this molecule intracellularly [169, 170]. More recent studies have revealed that HSV can also interfere with the expression of additional NK-activating ligands, such as ULBP1, ULBP2, and ULBP3 [171]. Importantly, HSVs can induce apoptosis in NK cells through Fas/FasL interaction between NK cells and HSV-infected macrophages, thus eliciting their deletion upon infection [121]. Although there is abundant evidence for negative modulation of NK cells by HSV, the contribution of these cells to HSV pathology remains somewhat controversial as both, negative and positive roles have been described for this cell population during HSV infection [172, 173].

Another innate immune cell type directly affected by HSV is natural killer T cells (NKT cells) (**Table 4**). These cells recognize glycolipid antigens presented on CD1d molecules [174]. Importantly, cells infected with HSV display reduced expression of CD1d on their surface, as they are redirected by viral determinants to intracellular compartments [175, 176]. Redirection of CD1d from the cell surface is mediated by the phosphorylation of host KIF3A by the viral kinase U_s3 [177]. Interestingly, vaginal application of α -galactosyl-ceramide an NKT ligand is shown to activate and recruit NKT cells to the genital tissue and decrease the susceptibility to HSV infection [178]. Remarkably, a recent report showed that NKT cells can contribute at determining the magnitude and profile of HSV-specific IgG antibodies upon HSV infection. HSV-infected NKT-cell-deficient mice displayed reduced amounts of antiviral IgM and IgG antibodies, as compared to wild-type mice [179]. These results suggest that NKT cells play an important role against HSV and that these viruses have evolved molecular mechanisms to interfere with their function. Furthermore, activating NKT cells with glycolipids may serve as a strategy to promote robust antibody responses against these viruses.

3.3. HSV interfere with dendritic cell function

Dendritic cells are immune cells strategically positioned at the interphase of innate and adaptive immunity. They are specialized in sensing microbes and danger signals, and also in integrating these signals and transducing them onto other cells for modulating the immune response to antigens [180-183]. Because DCs are key at connecting innate and adaptive immunity and clearing microbes, pathogens have evolved numerous immune evasion mechanisms to overcome their function [181, 184–189]. Importantly, HSV has been shown to infect DCs and to modulate their function by altering their maturation and capacity to activate T cells (Table 5) [118, 119, 190]. Furthermore, HSV can negatively modulate the autophagosome within DCs and interfere with their antigen processing capacity. This process is mediated by the viral protein γ 34.5, which blocks autophagosome maturation [191, 192]. On the other hand, HSV-2 protein ICP47 has been shown to specifically block the expression of particular alleles of MHC-I on the surface of human DCs, namely, HLA-C, potentially rendering these cells more susceptible to NK killing and reducing the spectrum of HSVderived antigens presented by these cells [193]. Remarkably, HSV has been shown to suppress many functions of DCs via caveolin-1 (Cav-1) by studying these cells in the lungs. HSVinduced Cav-1 was shown to downregulate the expression of inducible nitric oxide synthase; indeed, Cav-1-deficient mice or enhancement of nitric oxide production in wild-type mice ameliorated virus elimination and reduced pathology after HSV infection [194]. Furthermore, such crosstalk may occur between nonvirally infected dermal dendritic cells phagocytizing HSV-infected epidermal Langerhans cells, which are the first dendritic cells to encounter HSV in the skin [195].

Adaptive immune cell affected	Viral determinant	Outcome	Mechanism	References
Dendritic cell	Undetermined	Altered DC maturation and capacity to activate T cells	Undetermined	[118, 119, 190]
Dendritic cell	γ34.5	Interference with autophagosome function and hence antigen processing	Blocks autophagosome maturation	[191, 192]
Dendritic cell	ICP47	Increased susceptibility to NK attack	Blocks MCH expression (HLA-C allele)	[193]
Dendritic cell	HSV-induced Cav-1	Reduced virus elimination and increased pathology	Downregulates the expression of inducible nitric oxide synthase	[194]
Dendritic cell	Undetermined	Induces DC cell death	Undetermined	[118–120]
Humoral	gE	Blocks antibody function related to complement activation and antigen phagocytosis	Binding to the Fc portion of antibodies. Competes with C1q and FcγRs	[201, 202]
T cell	ICP47 and U _s 3	Reduced CTL recognition of infected cells and decreased naïve T cell activation	Interferes with host TAP protein, impairing peptide-MHC complex presentation	[205, 206]
T cell	gD, gB, gH, gI, and gL	Reduction in T cell activation and function. Decreases IL-2 secretion	Signals through HVEM. Alters CD3-dependent intracellular calcium signaling	[208]
T cell	U _s 3	Impairs T-cell activation	Interferes with TCR signaling. Blocks TRAF6 activity, altering LAT function	[209]
T cell	gD	Promotes Treg cell function with increased IL-10 secretion. Likely to alter CTL activity	Induces proliferation of T CD4 ⁺ FoxP3 ⁺ (CD25 ⁺) cell subsets	[213–217]

γ34.5 (late gene gamma 34.5, ICP34.5); ICP47 (infected cell protein 47); Cav-1 (caveolin-1); gB, gD, gE, gH, gI, gL (glycoproteins B, D, E, H, I, L); U_s3 (short unique region 3); DC (dendritic cell); NK (natural killer cell); Treg (regulatory T cell); MHC (major histocompatibility complex); HLA-C (human major histocompatibility complex chain C); C1q (subcomponent of the C1 complex of the classical pathway of complement activation); FcγR (Fc gamma receptor); TAP (transporter associated with antigen processing); HVEM (herpesvirus entry mediator); IL-2 (interleukin 2); CD3 (cluster of differentiation 3); TCR (T-cell receptor); TRAF3 (TNF receptor-associated factor 3); LAT1 (linker for activation of T cells); FoxP3 (forkhead box P3 protein).

Table 5. Evasion of adaptive immunity.

Importantly, experiments with animals depleted of DCs have shown that these cells are involved in neuron infection, as up to fivefold less latent virus can be found in the trigeminal ganglia of animals devoid of these cells [196]. Consistent with this notion, another study found that depletion of CD11c⁺ CD8 α^+ DCs reduced HSV latency in neurons after ocular infection and that Flt3L treatment, which increases the number of DCs in the tissues, enhanced virus infection of neurons [197]. These studies suggest that DCs may be used as Trojan horses by HSV to reach neurons or that the virus might manipulate these cells in such a way to gain access to the former. However, another study that assessed HSV infection through the footpad in the mouse model found that depletion of DCs was associated with increased viral loads in neurons [198].

Taken together, these studies evidence numerous evasion strategies evolved by HSV to alter the function of DCs and consequently innate and adaptive immunity (discussed below). Additionally, these viruses seem to have harnessed the mobile properties of DCs to spread onto other host cells and tissues, namely, neurons. The fact that HSVs ultimately induce DC apoptosis will likely interrupt the establishment of effective and robust immune responses against these viruses.

4. Herpes simplex viruses evade adaptive immunity

4.1. Interference with humoral immunity

Although natural infection with HSV elicits antiviral antibodies with *in vitro* neutralizing capacities, these responses seem largely insufficient in most individuals when it comes to limit HSV symptoms and virus shedding. This host antibody response is mostly directed to few surface viral antigens, mainly gD, gB, and, to a lesser extent, gC all of which are essential for virus entry, except for gC in HSV-2 [199]. For antibodies to exert effective antiviral activities they need not to be necessarily neutralizing, as antibodies can also elicit complement activation and immune complex-induced phagocytosis, thanks to their Fc portion [200]. However, HSVs have evolved molecular mechanisms to evade these antibody functions (Table 5). Notably, the HSV-encoded glycoprotein E (gE) can interfere with complement activation by directly binding to the Fc portion of antibodies and competing with complement component C1q [201, 202]. Indeed, gE functions as an IgG Fc receptor (FcyR) that binds the Fc domain of IgG antibodies and thus blocks their capacity to promote complement activation, altogether impeding phagocytosis by immune cells [202-204]. Importantly, specific interference with anti-HSV antibodies and not other circulating antibodies is achieved, thanks to the relatively low affinity of gE for the Fc portion of antibodies; antibodies are stabilized on the virus surface only if the Fab portion of the antibody is also bound to a viral antigen by its antigen-binding region. Hence, HSV has evolved molecular determinants to persist within the host and shed onto others, despite the existence of virus-neutralizing antibodies. Such evasion mechanisms have led to difficulties in the development of prophylactic formulations against HSV, and is further discussed below.

4.2. Evasion of T cell immunity

T cells can recognize microbe-derived protein fragments presented on the surface of infected cells and destroy these cells to limit virus replication and shedding onto other tissues and organisms. However, HSV encode molecular determinants that interfere with viral antigen presentation to T cells, namely, with MHC-I presentation, and thus the virus can hamper T-cell recognition of infected cells. HSVs interfere with the presentation of viral antigens by blocking the function of host TAP protein (transporter associated with antigen processing), which translocates self- and foreign peptides from the cytoplasm into the rough endoplasmatic reticulum for peptide loading onto MHC-I molecules (**Table 5**); TAP inhibition is mediated by the HSV protein ICP47 [205] and the U_s3 kinase [206]. Reduced peptide/MHC (pMHC) complexes on the surface of infected cells dramatically reduces the chances of cytotoxic T cells detecting HSV-infected cells, as well as the capacity of HSV-infected professional antigen presenting cells to activate naïve T cells.

Furthermore, additional mechanisms exist by which HSVs can negatively modulate the activation and proliferation of T cells (Table 5). For instance, the viral glycoprotein D binds to HVEM (herpesvirus entry mediator) on the surface of immune cells, which is a receptor belonging to the TNF-receptor superfamily and whose intracellular signaling mechanisms depends within others on the engagement of its different ligands and their orientation (cis vs. trans) [207]. gD binding to the cell surface of T cells has shown to alter calcium signaling within T cells after CD3 engagement, likely by interfering with the capacity of T-cell receptor to appropriately transduce intracellular signals that lead to suitable activation and function of these cells [208]. For instance, Jurkat T cells cultured with HSV and an activating CD3 antibody exhibit hampered IL-2 secretion [208]. A similar effect was observed with other HSV glycoproteins, namely, gB, gH, gI, and gL in the same study [208]. A recent report suggests that impaired T-cell activation would also be mediated by HSV U_s3 protein interference with T-cell receptor signaling, specifically by altering linker for activation of T cells (LAT1) within these cells [209]. Importantly, infection of T cells (other than Jurkat cells) requires the presence of antigen-presenting cells for efficient virus transfer, a process termed virological synapse. Indeed, primary cultures of T cells incubated with HSV alone are only infected at very low frequencies, while adding fibroblasts significantly enhances the formation of virological synapses that culminate in a substantial increase in the number of T cells infected with these viruses [210]. Importantly, HSV has been described to lead to T-cell apoptosis [211].

T cells can carry out numerous functions depending on their phenotype. While cytotoxic T cells are specialized in killing microbe-infected cells, regulatory T cells (Tregs) are specialized within others in controlling the magnitude of the immune response to antigens [212]. In this regard, HSV seems to promote the proliferation of regulatory T cells through the binding of gD to HVEM receptors on the cell surface to promote the secretion of signature cytokines attributed to these cells, such as IL-10 (**Table 5**) [213–216]. The promotion of Tregs might alter the activity of cytotoxic T cells intended to control the virus [213, 217]. Consistent with a negative role for Tregs in HSV infection, protection elicited against this virus in an animal model with previous immunization correlates with relatively low numbers of Tregs [218]. Nevertheless, another study proposes that deletion of Tregs in HSV-infected animals interferes

with the migration of immune cells to the site of infection, negatively affecting the survival of infected animals [219]. Thus, further studies are needed to determine the contribution of Tregs in HSV infection.

4.3. Past and present vaccine attempts

Availability of an effective vaccine against HSV would be an important public health advance, mainly because individuals with genital herpes display increased susceptibility to acquire HIV [141–145]. Importantly, previous efforts invested on the development of vaccines against HSV have concentrated on subunit approaches consisting mainly on one viral glycoprotein, namely, gD (Table 6) ([220], http://clinicaltrials.gov). Glycoprotein gD is conserved within HSV serotypes and plays a key role during cell infection [221]. Furthermore, this viral protein harbors epitopes for CD4⁺ T cells [222], CD8⁺ T cells [223], and neutralizing antibodies [224] and is immunodominant as evidenced by clinical data showing that the majority of HSVinfected individuals have neutralizing antibodies against this protein [199]. Regretfully, this insisted strategy, which combines gD with adjuvants, recently failed in a phase 3 clinical trial; indeed, the formulation failed at reducing both HSV-2 infection and minimizing the shedding of the virus [225, 226]. Remarkably, the formulation tested in this and previous clinical trials induced anti-gD neutralizing antibodies in the vaccinated, as well as T CD4⁺ cells [220, 227– 230]. However, the magnitude of these responses may have been too weak for significant protection against HSV-2 after exposure [199, 228, 231, 232]. Unexpectedly, the vaccine provided 35% cross-protection against HSV-1 infection and 58% cross-protection against HSV-1 disease [227]. An important concern that arose from these results was whether the current animal models used to assess the efficacy of new HSV vaccines satisfactorily recapitulate what occurs in humans. It is also unclear whether the amount and/or quality of neutralizing antibodies elicited against HSV and T cells produced by vaccine formulations, such as the glycoprotein D/AS04 vaccine, play any relevant role in protection against HSV-2; furthermore, whether previously considered correlates of protection as anti-gD antibodies play any relevant role against this virus.

Importantly, a recent study suggests that anti-HSV antibodies, different from those directed against gD, might account for effective protection against HSV-2 after immunization with a discontinuous virus (**Table 6**). Indeed, animals immunized subcutaneously with a genotypically deleted gD virus elicited remarkable protection against genital and skin challenge with HSV-1 and HSV-2, which was mediated by antibodies. Noteworthy, the antibodies elicited by this attenuated HSV strain were poorly neutralizing and were mainly directed against gB [233]. A somewhat similar result was found in another study with an 0 Δ NLS-attenuated HSV strain, which elicits antibodies against numerous virus-infected cell proteins (ICP) and gB, within others (**Table 6**) [234–236]. Other attenuated HSV strains have also provided promising results in numerous mouse models and should move onto clinical trials, such as the HSV-2 dl5-29 strain, which has U_L5 and U_L29 deleted from its genome (**Table 6**) [237–239]. Additional attenuated viral strains that confer significant protection against viral challenge are HSV strains that are impaired at infecting neurons, such as a gD mutant virus [240], HSV deleted at $U_L 39$ (ICP10 Δ PK) [241], and HSV deleted at gE (**Table 6**) [242]. Regretfully, an HSV strain

deleted at *gH*, which showed early promising results in animal models, was later shown to be ineffective in humans in a clinical trial [243]. Although most of these strategies elicit both anti-HSV antibodies and antiviral T cells, the main immune components involved in protection against HSV challenge remain unknown. Importantly, a recent study suggests that other animal models different from the guinea pig and the mouse infection model might be better suited for testing anti-HSV vaccine formulations. For instance, the cotton rat *Sigmodon hispidus* parallels well the results obtained with the D/AS04 vaccine in humans, both for HSV-1 and HSV-2 [244].

Formulation type	Outcome	Development stage	References
Subunit protein gD plus adjuvant Alum and MPL (gD/AS04)	Induces T CD4 ⁺ and antibody response. No clinical protection for shedding and infection.	Clinical phase 3 (completed)	[199, 220, 225– 233]
Live attenuated, HSV-2 virus with gH deletion (HSV-2 Δ gH)	Safe and immunogenic, yet did not confer protection to HSV infection.	Clinical phase 2 (completed)	[243]
Live attenuated, HSV-2 virus with U_L 39 deletion (ICP10 Δ PK)	Induction of Th1 immunity	Clinical phase 2 (completed)	[241]
Live attenuated, HSV-2 virus with $U_L 5$ and $U_L 29$ deletions (ACAM529)	Reduced disease, shedding, seroconversion, and latency	Preclinical stage	[237–239]
Live attenuated HSV-2 virus with gD mutation (HSV-2-gD27)	Protects from challenge and reduces viral load in neurons	Preclinical stage	[240]
Live attenuated HSV-2 virus with gD deletion (HSV-2 Δ gD ^{-/+})	Protects from genital and skin challenge and blocks neuronal infection. Antibody-mediated protection	Preclinical stage	[233]
Live attenuated, HSV-2 virus with gE deletion (HSV-2 Δ gE2)	Reduced infection and recurrence	Preclinical stage	[242]
Live attenuated HSV- 2 virus with ICP0 deletion (0ΔNLS)	Antibody response against gB and ICP viral proteins	Preclinical stage	[234–236]

AS04 (adjuvant system 04); MPL (monophosphoryl lipid A); gD, gE, gH (glycoprotein D, E, H); ICP10 Δ PK (infected cell protein 10 lacking the PK domain); Th1 (T helper-1); U_L5, U_L29, U_L39 (short unique region 5, 29, and 39); ICP0 (infected cell protein 0).

Table 6. Past and present vaccine attempts against HSV.

On the contrary to the evidence that suggests a role for antibodies in protection against HSV infection, a recent study proposes that effective protection against ocular HSV may be achieved by eliciting a robust T cell response alone. Indeed, humanized HLA transgenic animals

vaccinated with T cell epitopes from different viral proteins identified in asymptomatic individuals and combined with adjuvant was shown to confer protection against ocular herpes [245–247]. However, whether such results relate specifically to this type of herpetic disease or whether these T cells ultimately elicit an antibody response against HSV upon virus challenge remains unknown. Noteworthy, an important limitation of vaccine approaches that are based on one or few viral proteins is that only an oligoclonal set of T cells will be elicited, which may limit the effectiveness of formulation to a narrow set of individuals [248, 249].

Taken together, the HSV vaccine field has suffered an important failure and will need to revisit the immunobiology of its diseases. Importantly, the race for the development of novel prophylactic formulations against these viruses is reopened. While numerous groups aim at vaccine strategies that are based on defined viral proteins or viral epitopes, others propose attenuated HSV strains as an alternative for eliciting multiantigenic immune responses against these viruses. Regardless of the methods, a novel vaccine against HSV must guarantee safety for the immunocompetent and notably immunocompromised individuals. Remarkably, the lack of vaccines against HSV has encouraged considerable research in the field of microbicides, which might provide a strategy to prevent infection with these viruses [4].

5. Concluding remarks

Herpes simplex viruses have proven to be masters of immune evasion as they encode numerous molecular determinants that promote evasion of host sensing, signal transduction, cytokine secretion by immune and nonimmune cells, and, most importantly, interference with innate and adaptive immunity. These attributes likely explain the coexistence of HSV and humans since time immemorial and facilitates their high prevalence in the population [250]. Although HSV are seldom life threatening, the important economic burden they elicit with the diseases they produce and their association with HIV infection calls for the implementation of novel vaccines and improved treatments to stop their effects. Hopefully, lessons learned from past failed clinical trials will lead to novel strategies that will ultimately limit the impact of these viruses.

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