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Colonization of peripheral ganglia by herpes simplex virus type 1 and 2

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Herpes simplex virus type 1 (HSV-1) and 2 (HSV-2) infect and establish latency in neurons of the peripheral nervous system to persist lifelong in the host and to cause recurrent disease. During primary infection, HSV replicates in epithelial cells in the mucosa and skin and then infects neurites, highly dynamic structures that grow or retract in the presence of attracting or repelling cues, respectively. Following retrograde transport in neurites, HSV establishes latency in the neuronal nucleus. Viral and cellular proteins participate in the chromatinization of the HSV genome that regulates gene expression, persistence, and reactivation. HSV-2 modulates neurite outgrowth during primary infection and upon reactivation, probably to facilitate infection and survival of neurons. Whether HSV-1 modulates neurite outgrowth and the underlying mechanism is currently under investigation. This review deals with HSV-1 and HSV-2 colonization of peripheral neurons, with a focus on the modulation of neurite outgrowth by these viruses.

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Introduction

Herpes simplex virus type 1 (HSV-1) and HSV-2 are highly prevalent human pathogens, with worldwide infection rates in the human population of about 67% and 13%, respectively [1]. One of the most important features of these viruses is their ability to infect and establish latency in neurons of the peripheral nervous system of the sensory (e.g. trigeminal ganglia (TG); dorsal root ganglia (DRG)) and autonomic branches (e.g. superior cervical ganglia (SCG)) [2]. HSV causes a spectrum of diseases, including cold sores, genital herpes, herpes stromal keratitis, disseminated disease in the neonate, meningitis, and encephalitis [3]. Colonization and persistence in neurons, followed by reactivation and recurrent disease, are essential events of HSV pathogenesis.

During primary infection, HSV replicates productively in epithelial cells of the mucosa, skin, and cornea. To infect neurons, HSV reaches neurites — a term referring collectively to axons and dendrites — that innervate the periphery. Neurites are not static entities, they can grow or retract, depending on the presence of attracting or repelling cues, respectively [4–6]. Epithelial cells in the mucosa and skin can release proteins that affect neurite outgrowth and neuronal survival [4,7–9].

HSV cell entry and delivery of the viral genome to the cell nucleus is a complex process that has been recently reviewed [10]. Upon delivery of the viral genome to a non-neuronal nucleus, a cascade of gene expression starts with the transcription of immediate early (IE) genes, followed by that of early and late genes, leading to production of infectious viral particles. Expression of IE genes requires the formation of a complex between the tegument protein VP16, host cell factor 1, and octamer-binding protein-1 [11]. However, the default outcome of neuronal infection *in vivo* is latency, characterized by the presence of a chromatinized, circularized viral genome that persists as an episome [12], and by restricted viral gene expression [13]. The main transcripts found in human TG obtained at short post-mortem interval correspond to the latency-associated transcript (LAT) and microRNAs [14].

It is not fully understood which properties make neurons suitable for HSV latency compared with other cells, but neuronal polarization seems relevant. Upon delivery of the capsid to the cytoplasm, several tegument proteins, including VP16, dissociate from the capsid and are transported to the cell nucleus independently of the capsid. Tegument proteins reach the nucleus later than the genome due to the long distance from the neurite end [15], failing to initiate IE gene expression in most neurons. Infection of neurons *in vitro* leads to lytic replication and production of infectious viral particles, unless the

virus is repressed through addition of drugs such as acyclovir or interferon (IFN), or if infection occurs through the neurite end [16–19]. Indeed, the combination of low multiplicity of infection with cell culture devices that separate neuronal somata and neurites, showed that infection through the cell body normally leads to lytic replication, while infection through the neurite end tends to result in latency [19,20]. Experiments performed with Campenot chambers and chicken TG support that inefficient transport of tegument proteins to the nucleus contributes to latency [19]. Infection at the neurite end leads to a phenotype similar to latent infection, unless this is combined with expression of VP16 in the cell body, a situation that results in productive infection [19]. Koyuncu and colleagues also found that complementation of pseudorabies virus neurite infection with tegument proteins in the cell body inhibited virus repression, leading to productive infection [21].

Chromatinization of the epigenetically naive HSV genome, imposed by cellular proteins and enhanced by LAT, regulates viral gene expression [22–25]. Among the cellular proteins and protein complexes involved are the repressor element 1 (RE1)-silencing transcription factor (REST)/CoREST/histone deacetylases, IFN-inducible protein 16, the polycomb repressive complex, promyelocytic leukemia nuclear bodies, and histone H3.3 chaperone complexes [22,23,26–28]. These proteins could be considered as part of the cellular intrinsic response to HSV infection. Some viral proteins, including ICP0, efficiently inhibit this response in non-neuronal cells [29,30].

During latency, most viral genes contain marks of facultative or repressive heterochromatin (e.g. H3K27me3 and H3K9me3), while the LAT locus has euchromatin modifications (H3K9ac and H3K14ac) that facilitate transcription [24,31]. The LAT promoter also contains a neuron-specific enhancer that increases its expression [32]. Chromatin insulators separate transcriptionally active and repressed genomic regions and facilitate establishment of latency by modulating deposition of heterochromatin [33]. Among the relevant functions of LAT during latency are the inhibition of neuronal apoptosis and the repression of lytic gene transcription [24,25,34]. Viral microRNAs located in the LAT locus also contribute to latency by targeting IE genes and ICP34.5 [35–37].

Even though neuronal infection *in vivo* normally leads to latency, lytic gene expression has been observed in some neurons in murine models [38,39]. The expression of IE gene ICP0 was suggested to initiate transcription of LATs during establishment and maintenance of latency [40], but how this lytic gene expression starts is not fully understood. *De novo* neuron-specific VP16 gene expression through Early Growth Response Protein-1 (Egr-1)/promoter specificity protein 1(Sp1)-binding sites located in its promoter could trigger expression of HSV IE genes [41,42]. Whether the detected expression of lytic genes corresponds to reactivation events or to a gene expression program before or during latency is not currently known. For excellent reviews on this topic and on reactivation see [43,44].

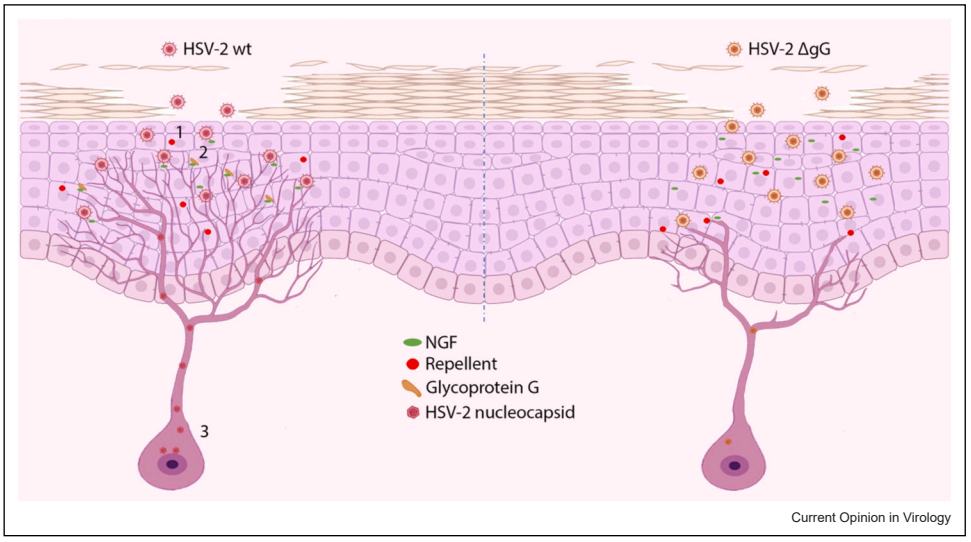
Following reactivation, HSV-1 and HSV-2 particles travel in an anterograde manner toward the neurite end, which can innervate the periphery or synapse with neurons of the central nervous system (CNS). Transport toward the periphery leads to lytic infection in the skin, mucosa, or cornea, facilitating recurrent disease and transmission to a new host. Transport toward the CNS could result in neuroinvasion and severe disease, including lethal encephalitis. Neurites are therefore key for HSV pathogenesis during primary infection and upon reactivation. The following sections focus on the impact of HSV infection on neurite outgrowth and survival.

Herpes simplex virus type 2 glycoprotein G binds and enhances the activity of the neurotrophin nerve growth factor

During primary infection, HSV replicates productively in epithelial cells of the epidermis and mucosa producing virions that infect neurites to colonize peripheral ganglia. Both sensory and autonomic nerve endings innervate the epidermis and dermis, while encapsulated corpuscles are more common in the dermis, with the exception of Merkel disks. The mucosa also contains bulbous corpuscles that sense cold [45]. The vaginal mucosa contains more innervation in the lamina propria and the distal third of the anterior vaginal wall than in other regions [46]. For a detailed review on the innervation of the skin and genital mucosa, see [45,46]. Epithelial and immune cells secrete proteins that modulate neurite outgrowth and neuronal survival, including neurotrophic factors, semaphorins, and cytokines [47–51]. There are two main families of neurotrophic factors: the neurotrophins and the glial cell line-derived neurotrophic factor family of ligands (GFL) [52,53]. Other proteins collectively termed axon guidance molecules (AGMs), include members of the semaphorin, ephrin, etrin, and slit families that induce or inhibit neurite outgrowth [4,54,55].

HSV infection modifies the expression profile of the skin and mucosa and triggers infiltration of immune cells [8,56,57]. Changes in the level of cytokines, neurotrophic factors, and AGMs, together with the infiltration of immune cells, could impact neurite outgrowth and survival and affect HSV neuroinfection. Since infection of neurites is essential for HSV persistence, we hypothesized that HSV-1 and HSV-2 developed strategies to regulate neurite outgrowth facilitating infection of neurons and their survival.

Figure 1



Proposed model for HSV-2 glycoprotein G activity. Schematic representation of the infection of the skin with wild-type HSV-2 (left) and HSV-2 lacking gG (right). During primary HSV-2 infection in the skin, there is secretion of neurotrophic factors such as NGF and repellents of neurite outgrowth (1). gG binds NGF and increases its activity, leading to higher neurite outgrowth (2) and, potentially, to neuronal survival. This could facilitate neuronal infection, increasing the number of infected neurons (3). Based on [58,69]. Partially created with Biorender.

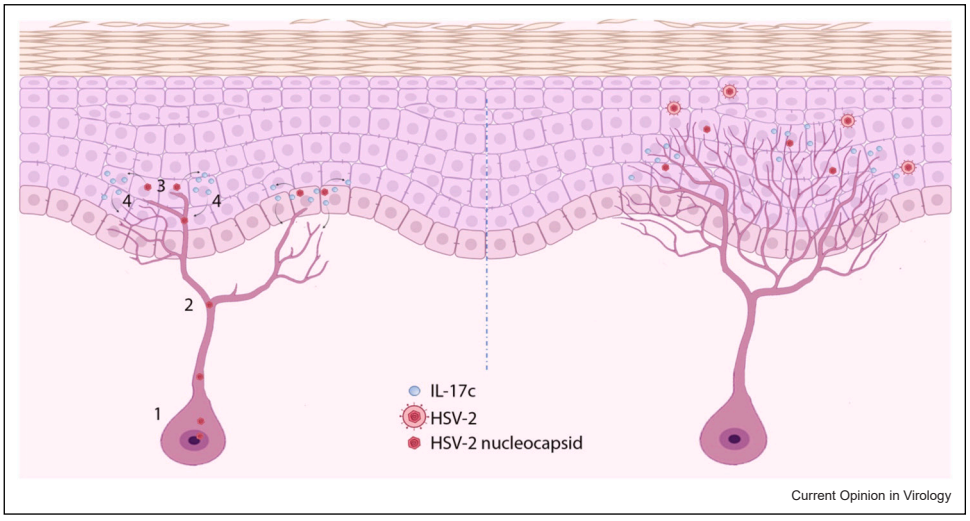
Our initial attempts focused on determining whether viral glycoproteins modulate the activity of neurotrophic factors, proteins that induce neurite outgrowth and neuronal survival [52,53]. We found that HSV-1 and HSV-2 glycoprotein G (gG1 and gG2, respectively) bind several neurotrophins, including nerve growth factor (NGF), and GFL members such as artemin [58]. gG1 and gG2 are the most divergent glycoproteins between HSV-1 and HSV-2, and this difference allows to distinguish between these viruses in diagnostic assays [59]. Both gG1 and gG2 are type-I transmembrane proteins. However, gG2 is cleaved at residues 318 and 341 by a furin-like protease, leading to secretion of the N-terminal domain, while gG1 is not [60–63]. The functional significance of this difference between both proteins is currently unknown. Interestingly, the secreted domain of gG2 binds to glycosaminoglycans allowing interaction with the plasma membrane and probably with the viral envelope [64,65]. HSV-1 and HSV-2 lacking gG expression replicate similarly to their corresponding parental viruses, indicating that these proteins are not essential for infection and replication *in vitro* [66–69]. However, gG could contribute to pathogenesis in animal models, including neuroinvasion [68].

We further explored the interaction between the ectodomain of gG1, the secreted domain of gG2, and neurotrophins and showed that gG2 enhances the activity of NGF, but not that of artemin [58]. This resulted in higher neurite outgrowth of mouse SCG neurons *ex vivo*. Interestingly, gG1 did not have any effect on NGF or artemin activities, despite binding to these neurotrophic factors with high affinity [58]. HSV primary infection often occurs in the orolabial surface, providing access to SCG and TG neurons innervating this anatomical area. Infection of mouse footpad with HSV-2 also increases neurite outgrowth [58]. We hypothesize that the increased neurite outgrowth could facilitate infection of neurites and neuronal survival (Figure 1).

NGF binds to two receptors: (TrkA) and P75 neurotrophin receptor (P75NTR) [53,70]. TrkA binding normally results in activation of signaling cascades, leading to neurite outgrowth and neuronal survival, while interaction with the low-affinity receptor p75NTR has many consequences ranging from apoptosis induction to an increase in TrkA activity [71]. The mechanism of action of gG2 involves the modulation of TrkA biology, including its increased phosphorylation, and an impairment of its retrograde transport. We addressed whether gG2 interacted with TrkA and detected a complex between gG2–NGF–TrkA, but not in the absence of the neurotrophin [58].

NGF is also required for the development and maintenance of nociceptive neurons [72] that mediate pain, and the NGF/TrkA axis regulates the biology of vanilloid receptor 1 tropomyosin receptor kinase A (TRPV1), a nociceptor involved in the sensation of heat and painful stimuli [73]. Interestingly, gG2–NGF interaction increases the phosphorylation of TRPV1 in mouse DRG neurons and injection of gG2 in the mouse

Figure 2



HSV-2 induces neurite outgrowth and neuronal survival following reactivation in sacral ganglia. Left: HSV-2 reactivates in sacral ganglia (1). HSV-2 particles travel anterogradely toward the epidermis (2), where HSV-2 infects keratinocytes and induces the expression of IL-17c (3). This cytokine binds to its receptor on neurite ends (4), increasing neurite outgrowth and neuronal survival. Right: Representation of the increased neurite outgrowth observed in genital skin after HSV-2 reactivation. Based on the analysis of genital skin biopsies obtained from individuals with HSV-2 reactivation [8]. Partially created with Biorender.

footpad lowered the threshold for heat-induced pain [74]. Whether this mechanism plays a role in the induction of pain by HSV-2, as occurs during genital herpes, is not known and requires further investigation.

Interestingly, we had previously shown that gG1 and gG2 bind chemokines with nanomolar affinity and enhance chemokine-dependent migration [75]. Chemokines are chemotactic cytokines that orchestrate leukocyte migration during development, homeostasis, inflammation, and infection, playing key roles in the antiviral response [76]. Several poxviruses and herpesviruses express proteins that bind and modulate chemokine activity [76]. HSV virions containing gG also bind chemokines and enhance chemotaxis [65]. Interestingly, chemokines can also induce neurite outgrowth and pain [77]. Whether the modulation of chemokine activity by gG1 and gG2 affects these two processes is currently unknown.

Herpes simplex virus type 2 infection of non-neuronal cells reduces their repulsion on neurite outgrowth

The experiments showing that gG2 binds and enhances NGF activity were performed mainly with purified recombinant gG2 [58]. To investigate whether gG2 could modulate neurite outgrowth during infection, we generated recombinant HSV-2 lacking gG2 expression and established an *ex vivo* model with microfluidic devices to study the impact of conditioned medium from non-neuronal cells, including epithelial cells, on neurite outgrowth [69]. Several cell types, including human embryonic kidney 293T (HEK-293T) and epithelial spontaneously arising retinal pigment epithelia (ARPE19) cells, express repellents of neurite outgrowth, potentially members of the AGM, that inhibit neurite outgrowth of peripheral neurons. Infection of these non-neuronal cells with HSV-2 overcomes their repulsion, leading to higher number and longer neurites that reach infected HEK-293T cells [69]. Infection with HSV-2 lacking gG resulted in lower number and shorter neurites than with the parental virus. Both NGF and gG2 play a role in this process, although it is possible that other viral proteins could modulate the expression of AGMs and neurotrophic factors, facilitating neurite outgrowth. For instance, HSV infection normally leads to reduction of cellular gene expression, mainly through the action of the virus host-shutoff protein (VHS), infected cell protein 4 (ICP4), and ICP27 [78–81]. Therefore, it is reasonable to hypothesize that viral proteins such as VHS, could inhibit the expression of repellents of neurite outgrowth.

New results from ongoing projects in our lab show that HSV-1 infection of epithelial cells also reduces their repelling effect, while an HSV-1 mutant lacking gG1 expression does not do so efficiently. The role of gG1 in this process is further supported by the fact that exogenous gG1 expression in epithelial cells leads to similar reduction of repulsion of neurite outgrowth. Interestingly, HSV-1 seems to increase neurite outgrowth by modifying the protein composition of extracellular vesicles rather than by increasing NGF activity.

Herpes simplex virus type 2 increases neurite outgrowth upon reactivation in human sacral ganglia

Following genital infection, HSV-1 and HSV-2 can establish latency in human sacral DRG and, upon reactivation, they travel in an anterograde manner to the genital mucosa and skin where they infect keratinocytes. The group of Jia Zhu observed higher number of neurites in biopsies of human skin following HSV-2 reactivation than in non-reactivated tissue. They then performed laser capture microdissection of keratinocytes followed by analysis of their transcriptional profile and identified interleukin-17c (IL-17c) as a potential protein involved in neurite outgrowth. *In vitro* experiments showed that infection of primary human keratinocytes with both HSV-1 and HSV-2 induced the expression of IL-17c, probably through the action of ICP0. They also showed that IL-17c acts as a neurotrophic factor, increasing neurite outgrowth of sensory neurons [8] (Figure 2). This is a clear demonstration that HSV-2 induces neurite outgrowth of human peripheral neurons upon reactivation *in vivo*. Interestingly, IL-17c also reduced apoptosis of mouse primary cortical neurons, leading the authors to suggest that it would also increase neuronal survival in a situation of recurrent reactivation [8]. Whether HSV-2 induces IL-17c expression during primary infection to promote neurite outgrowth and neuronal infection is an intriguing question, although this is technically difficult to investigate since primary infection of HSV is mostly asymptomatic and unrecognized. Moreover, whether HSV-1 reactivation leads to neurite outgrowth has not been shown. However, the fact that HSV-1 infection of primary keratinocytes induces the expression of IL-17c [8] supports this hypothesis.

Conclusion

Despite the importance for the viral replication cycle, the mechanisms by which HSV ensures the effective infection of neurites and neuronal survival are not understood yet. HSV-1 and HSV-2 infection of epithelial cells overcomes their repulsion on neurite outgrowth. In the case of HSV-2, our results point to the interaction between gG2 and NGF as a key factor [58,69]. Moreover, HSV-2 infection of human keratinocytes increases the expression of IL-17c and, thereby, neurite outgrowth [8]. This leads to reduced neuronal apoptosis, probably increasing neuronal survival and HSV-2 persistence during frequent HSV-2 reactivation. In the case of HSV-1, our recent findings suggest that this virus enhances neurite outgrowth by modifying the composition of extracellular vesicles (unpublished results). Research in this field could provide mechanisms to inhibit HSV infection of neurons and tools to improve neurite outgrowth and neuronal regeneration in neurological disorders.

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Data Availability

No newly generated data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- •• of outstanding interest

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